FORM-PTO-1390 (Rev. 12-29-99) DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER: 001560-397

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): To be assigned 09/830123

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP00/05722	24 August 2000	24 August 1999

TITLE OF INVENTION
GENES ENCODING PROTEINS REGULATING THE pH OF VACUOLES

APPLICANT(S) FOR DO/EO/US
Shigeru IIDA, Sachiko TANAKA, and Yoshishige INAGAKI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    International Search Report
    Sequence Listing (paper copy)
    Japanese PCT Request Form
    PCT Notice Informing the Applicant of the Communication of the International Application to the Designated Offices (Form PCT/IB/308)
    Cover page from published PCT international application (WO 01/14560)

JC18 Rec'd PCT/PTO 24 APR 2001

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
To be assigned 09/830123	PCT/JP 00/05722	001560-397

	CALCULATIONS	PTO USE ONLY
17. ☐ The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00 (960)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00 (970)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00 (958)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00 (956)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00 (962)		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00 (154)** for furnishing the oath or declaration later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	51 -20 =	31	X$18.00 (966)	$ 558.00	
Independent Claims	3 -3 =	0	X$80.00 (964)	$	
Multiple dependent claim(s) (if applicable)			+$270.00 (968)	$	
TOTAL OF ABOVE CALCULATIONS =				$	
Reduction for 1/2 for filing by small entity, if applicable (see below).				$	-
SUBTOTAL =				$ 1,418.00	
Processing fee of **$130.00 (156)** for furnishing the English translation later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 1,418.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 (581)** per property +				$ 40.00	
TOTAL FEES ENCLOSED =				$ 1,458.00	
				Amount to be: refunded	$
				charged	$

a. ☐ Small entity status is hereby claimed.

b. ☒ A check in the amount of $ 1,458.00 to cover the above fees is enclosed.

c. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Ronald L. Grudziecki
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Michael K. Mayo #39,300
SIGNATURE

for Donna M. Meuth
NAME

36,607
REGISTRATION NUMBER

April 24, 2001

(01/01)

Patent
Attorney's Docket No. 001560-397

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
Shigeru IIDA et al	)	Group Art Unit: To be assigned
	)	
Application No.: To be assigned	)	Examiner: To be assigned
(National Stage of PCT International Appln.	)	
No. PCT/JP00/05722 filed August 24, 2000)	)	
	)	
Filed: April 24, 2001	)	
	)	
For: GENES ENCODING PROTEINS REGULATING THE pH OF VACUOLES	)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application on the merits, please amend the application as follows:

IN THE SPECIFICATION

Kindly replace the paragraph beginning at page 5, line 15, with the following:

-- The present invention also provides a plant in which said gene or said vector has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.--

Kindly replace the paragraph beginning at page 5, line 19, with the following:

-- The present invention also provides a cut flower of the above plant or a progeny thereof.--

Please add the paper copy of the Sequence Listing included herewith to the application, after page 19 and before the Claims on page 20.

Please renumber the pages accordingly.

IN THE CLAIMS

Please replace claims 7, 9, and 11-14 as follows:

7. (Amended) A vector comprising the gene according to claim 1.

9. (Amended) A protein encoded by the gene according to claim 1.

11. (Amended) A plant in which the gene according to claim 1 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.

12. (Amended) A cut flower of the plant according to claim 11 or a progeny thereof having the same property as said plant.

13. (Amended) A method of regulating the pH of vacuoles comprising introducing the gene according to claim 1 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

14. (Amended) A method of controlling the flower color of a plant comprising introducing the gene according to claim 1 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

Please add new claims 15-51 as follows:

--15. A vector comprising the gene according to claim 2.

16. A vector comprising the gene according to claim 3.

17. A vector comprising the gene according to claim 5.

18. A vector comprising the gene according to claim 6.

19. A host cell transformed with the vector according to claim 15.

20. A host cell transformed with the vector according to claim 16.

21. A host cell transformed with the vector according to claim 17.

22. A host cell transformed with the vector according to claim 18.

23. A protein encoded by the gene according to claim 2.

24. A protein encoded by the gene according to claim 3.

25. A protein encoded by the gene according to claim 5.

26. A protein encoded by the gene according to claim 6.

27. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 19 and then harvesting said protein from said host cell.

28. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 20 and then harvesting said protein from said host cell.

29. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 21 and then harvesting said protein from said host cell.

30. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 22 and then harvesting said protein from said host cell.

31. A plant in which the gene according to claim 2 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.

32. A plant in which the gene according to claim 3 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.

33. A plant in which the gene according to claim 5 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.

34. A plant in which the gene according to claim 6 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.

35. A cut flower of the plant according to claim 31 or a progeny thereof having the same property as said plant.

36. A cut flower of the plant according to claim 32 or a progeny thereof having the same property as said plant.

37. A cut flower of the plant according to claim 33 or a progeny thereof having the same property as said plant.

38. A cut flower of the plant according to claim 34 or a progeny thereof having the same property as said plant.

39. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 2 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

40. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 3 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

41. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 5 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

42. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 6 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

43. A method of controlling the flower color of a plant comprising introducing the gene according to claim 2 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

44. A method of controlling the flower color of a plant comprising introducing the gene according to claim 3 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

45. A method of controlling the flower color of a plant comprising introducing the gene according to claim 5 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

46. A method of controlling the flower color of a plant comprising introducing the gene according to claim 6 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

47. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 1 in a plant or plant cells.

48. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 2 in a plant or plant cells.

49. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 3 in a plant or plant cells.

50. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 5 in a plant or plant cells.

51. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 6 in a plant or plant cells.--

REMARKS

Prior to examination, entry of the foregoing is respectfully requested.

Claims 7, 9, and 11-14 have been amended simply to delete multiple dependencies in the claims and correct claim dependencies. Minor amendments relating to matters of form only have also been made.

New claims 15-51 have been added, directed to preferred embodiments of the invention in view of the deletion of multiple dependent claims. Support for these additional claims may be found at the very least in original claims 1-14 and at page 19, lines 12-24. No new matter has been added.

In the event that there are any questions relating to this Preliminary Amendment, or to the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney at (508) 339-3684 concerning such questions so that prosecution of this application may be expedited.

Early and favorable action in the form of a Notice of Allowance is respectfully requested and believed to be in order.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: Donna M. Meuth #39,300
for Donna M. Meuth
Registration No. 36,607

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Date: April 24, 2001

Attachment to Preliminary Amendment dated April 24, 2001

Marked-up Copy

Page 5, Paragraph Beginning at Line 15

The present invention also provides a plant in which said gene or said vector has been introduced or [an] a progeny thereof having the same property as said plant, or a tissue thereof.

Attachment to Preliminary Amendment dated April 24, 2001

Marked-up Copy

Page 5, Paragraph Beginning at Line 19

The present invention also provides a cut flower of the above plant or [an] a progeny thereof.

Attachment to Preliminary Amendment dated April 24, 2001

Marked-up Claims 7, 9, and 11-14

7. (Amended) A vector comprising the gene according to claim 1 [any one of the claims 1 to 6].

9. (Amended) A protein encoded by the gene according to claim 1 [any one of claims 1 to 6].

11. (Amended) A plant in which the gene according to claim 1 [any one of claims 1 to 6 or the vector according to claim 7] has been introduced or a [an] progeny thereof having the same property as said plant, or a tissue thereof.

12. (Amended) A cut flower of the plant according to claim 11 or a [an] progeny thereof having the same property as said plant.

13. (Amended) A method of regulating the pH of vacuoles comprising introducing the gene according to claim 1 [any one of claims 1 to 6 or the vector according to claim 7] into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

14. (Amended) A method of controlling the flower color of a plant [plants] comprising introducing the gene according to claim 1 [any one of claims 1 to 6 or the vector according to claim 7] into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

09/830123
JC18 Rec'd PCT/PTO 24 APR 2001
STY-H794

DESCRIPTION

GENES ENCODING PROTEINS REGULATING THE pH OF VACUOLES

Technical Field

The present invention relates to genes encoding proteins that regulate the pH of vacuoles, and the uses thereof.

Background Art

In the flower industry, the development of novel or varied cultivars of flowering plants is important, and flower color is one of the most important traits of flowers. Although cultivars of various colors have been bred using conventional breeding by crossing, it is rare that a single plant species has cultivars of all colors. Thus, there is a need for the development of cultivars having a variety of colors.

The main components of flower color are a group of flavonoid compounds termed anthocyanins. It is known that a variety of anthocyanins occur in plants, and the structure of many of them have already been determined. The color of anthocyanins depends partly on their structures. Progress has been made in the study on the enzymes and genes involved in the biosynthesis of anthocyanins, and in some studies molecular biological techniques and gene introductions into plants were used to change the structure of anthocyanins, leading to changes in the color of flowers (Holton and Cornish, Plant Cell, 7:1071 (1995); Tanaka et al., Plant Cell Physiol. 39:1119 (1998)). The color of anthocyanins also depends on the pH of the aqueous solution, and the same anthocyanin may appear blue when the pH of the aqueous solution is neutral to weakly alkaline (Saito and Honda, Genda Kadaku (Chemistry Today), May 1998, pp. 25).

It is also known that since anthocyanins are present in the vacuole of the cell, the pH of vacuoles has a

great impact on the color of flowers (Holton and Cornish, Plant Cell, 7 (1995); Mol et al., Trends Plant Sci. 3:212 (1998)). For example, in morning glory (Ipomea tricolor), it is known that the reason why red-purple buds bloom into blue flowers is that the pH of vacuoles in petal epithelium rises from 6.6 to 7.7 (Yoshida et al., Nature 373:291 (1995)).

It is thought that the vacuole of plant cells is regulated by vacuolar proton-transporting ATPase and vacuolar proton-transporting pyrophosphatase (Leigh et al., The Plant Vacuole (1997), Academic Press), but the mechanism of how these proton pumps are involved in the color of flowers has not been elucidated. It was also known that a sodium ion-proton antiporter (hereinafter referred to as Na^+-H^+ antiporter) exits in plant vacuoles and that the Na^+-H^+ antiporter transports sodium ions into vacuoles, depending on the proton concentration gradient between the outside and the inside of vacuoles, whereupon protons are transported outside of vacuoles resulting a reduced proton concentration gradient.

Furthermore, the Na^+-H^+ antiporter is thought to be a protein with a molecular weight of about 170,000. However, there are many unknown factors involved in the regulation of pH of vacuoles, and the mechanism of regulating the pH of vacuoles, in particular the petal vacuoles, is uncertain (Leigh et al., The Plant Vacuole (1997), Academic Press). The pH of plant vacuoles has never been artificially raised, nor have any industrially useful traits been obtained, and its association with flower color is unknown.

It is known that the Na^+-H^+ antiporter gene, with a molecular weight of about 70,000, has been cloned from Arabidopsis, and a yeast into which this gene was introduced has acquired salt tolerance (Gaxiola et al., Proc. Natl. Acad. Sci. USA 96:1480-1485 (1999)), but it is not known how this antiporter regulates the pH of vacuoles in plant cells or how it is associated with

flower color.

On the other hand, in petunias, seven loci are known to be involved in the pH regulation of petal vacuoles, and it has been proposed that the pH of petal vacuoles increases when one of them turns homozygously recessive (van Houwelingen et al., Plant J. 13:39 (1998); Mol et al., Trends Plant Sci. 3:212 (1998)). One of them, Ph6, has already been cloned and was found to be a kind of transcription regulating factor (Chuck et al., Plant Cell 5:371 (1993)), but the actual biochemical mechanism involved in the pH regulation of vacuoles is unknown.

In morning glory (Ipomea nil), the analysis of mutants revealed that a number of loci are associated with the color and shape of leaves and flowers and that 19 of them are highly mutable (Iida et al., Shokubutsu Saibo Kogaku Series (Plant Cell Engineering Series) 5 (1996) pp. 132, Shujunsha; Iida et al., Annal. New York Acad. Sci. (1999) pp. 870). Among them, the one locus defined by the recessive mutation that results in purple flowers instead of blue flowers is termed the Purple locus (T. Hagiwara, The genetics of flower colours in Phrarbitis nil. J. Coll. Agr. Imp. Univ. Tokyo 51:241-262 (1931); Y. Imai, Analysis of flower colour in Pharbitis nil. J. Genet. 24:203-224 (1931)), and one allele of mutable mutation that results in flowers that produce blue sectors in purple petals was termed purple-mutable (pr-m) (Imai, J. Coll. Agric. Imp. Univ. Tokyo 12:479 (1934)). The gene derived from the Purple locus is termed Purple gene.

The blue portion is believed to be derived from somatic reverse mutation from the recessive purple, and germ cell revertants can also be separated. An allele produced from the reverse mutation of these revertants are termed herein Purple-revertant (Pr-r). Such a classical method of genetic analysis had been performed on this Purple gene, but the identity of the Purple gene and its association etc. with the pH regulation of petal

vacuoles were totally unknown.

It is believed that if the pH of vacuoles could be modified, for example if the pH of vacuoles could be raised, flower color could be turned blue. Representative plant species that lack blue colors include roses, chrysanthemums, carnations, gerberas and the like, which are very important cut flowers. Though the importance of modifying pH of vacuoles has been recognized, the identities of proteins that regulate the pH of petal vacuoles are unknown and therefore the isolation of genes encoding them has been in great demand.

Disclosure of the Invention

The present invention provides a gene of a protein that regulates the pH of vacuoles in plant cells, preferably a gene of a protein that transports protons in vacuoles, more preferably a Na^+-H^+ antiporter gene. By introducing the gene of the present invention into a plant and allowing it to be expressed, flower color can be controlled and, preferably, can be turned blue.

Thus, the present invention provides a gene encoding a protein that regulates the pH of vacuoles. This gene is, preferably, a gene encoding a Na^+-H^+ antiporter, for example a gene encoding a protein that has the amino acid sequence as set forth in SEQ ID NO: 2, or a gene encoding a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other amino acids in the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles; a gene encoding a protein that has an amino acid sequence having a identity of 20% or more with the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles; or, a gene that hybridizes to part or all of a nucleic acid having a nucleotide sequence encoding the amino acid sequence as set forth in

SEQ ID NO: 2 under a stringent condition, and that encodes a protein having an activity of regulating the pH of vacuoles.

The present invention also provides a vector comprising the above gene.

The present invention also provides a host cell transformed with the above vector.

The present invention also provides a protein encoded by the above gene.

The present invention further provides a method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the above host cell and then harvesting said protein from said host cell .

The present invention also provides a plant in which said gene or said vector has been introduced or an progeny thereof having the same property as said plant, or a tissue thereof.

The present invention also provides a cut flower of the above plant or an progeny thereof.

The present invention further provides a method of regulating the pH of vacuoles comprising introducing the above gene or the above vector into a plant or plant cells and then allowing it to be expressed.

The present invention further provides a method of controlling the flower color of plants comprising introducing the above gene or the above vector into a plant or plant cells and then allowing said gene to be expressed.

Brief Explanation of the Drawings

Fig. 1 is a drawing showing the structure of plasmid pSPB607.

Fig. 2 is a drawing showing the structure of plasmid pSPB608.

Fig. 3 is a drawing showing the structure of plasmid pINA145.

Fig. 4 is a drawing showing the structure of plasmid pINA147.

Best Mode for Carrying Out the Invention

The color of the petal of morning glory is blue when the locus Purple is dominant, and the blue petal turns purple when it is homozygously recessive. It is clear that the locus is associated with flower color but the mechanism thereof is unknown.

First, the chemical analysis of the pigments in the petal of the pr-m mutant and a revertant thereof detected no difference in the composition of the pigments. The change in flower color of the blue-colored morning glory from the reddish purple buds to the blue flowers accompanied by flowering is believed, as mentioned above, to be caused by pH changes in the vacuole of petal cells.

In the pr-m mutant, flowering is not associated with a color change to blue, and the pH of vacuoles of petal cells of flowers that bloomed was lower in the pr-m mutant than in Pr-r. Thus, the Purple gene is considered to be a gene that regulates the pH of vacuoles of petal cells during flowering and thereby controls flower color. Accordingly, using a pr-m mutant, and a revertant thereof, by the transposon display method, fragments of genomic DNA containing the Purple gene sequence specifically present in pr-m were identified and then the Purple gene was identified. Surprisingly, the Purple gene thus obtained had a homology with the Na^+-H^+ antiporter from Arabidopsis etc., and, in the pr-m mutation, a transposon had been inserted in the 5'-untranslated region the Purple gene.

As the gene of the present invention, there can be mentioned, for example, one that encodes the amino acid sequence as set forth in SEQ ID NO: 2. It is known, however, that proteins having an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other

amino acids also retain an activity equal to that of the original protein. Thus in accordance with the present invention, a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other amino acids in the amino acid sequence as set forth in SEQ ID NO: 2, and a gene encoding said protein, are encompassed in the present invention as long as the protein is a protein that has an activity of regulating the pH of vacuoles.

The present invention also relates to a gene that hybridizes to the nucleotide sequence as set forth in SEQ ID NO: 1, a nucleotide sequence encoding the amino acid sequence as set forth in SEQ ID NO: 2, or a nucleotide sequence encoding part of these nucleotide sequences at a stringent condition, for example at 5 × SSC and 50°C, and that encodes a protein having an activity of regulating the pH of vacuoles. As used herein, a suitable hybridization temperature varies with the nucleotide sequence and the length of the nucleotide sequence, and when, for example, a DNA fragment comprising 18 bases encoding 6 amino acids is used as a probe, a temperature of 50°C or lower is preferred.

Genes selected, based on such hybridization, include those obtained from nature, for example from plants such as petunia and torenia, but a gene derived from sources other than plants may be used. Genes selected based on hybridization may be cDNA or genomic DNA.

The Na^+-H^+ antiporter genes form a superfamily (Debrov et al., FEBS Lett. 424:1 (1998)), and have an amino acid homology of 20% or more (Orlowski et al., J. Biol. Chem. 272:22373 (1997)).

Thus, the present invention relates to a gene encoding a protein that has an amino acid sequence with a homology of about 20% or more, preferably 50% or more, for example 60% or 70% or more, and that has an activity of regulating the pH of vacuoles.

A gene having an intact nucleotide sequence is obtained, as specifically illustrated in Examples, by, for example, screening cDNA libraries. DNA encoding a protein having a modified amino acid sequence can be synthesized by commonly used site-directed mutagenesis or the PCR method based on DNA having an intact nucleotide sequence. For example, a DNA fragment that is to be modified may be obtained by restriction enzyme treatment of the intact cDNA or genomic DNA, which is used as a template in the site-directed mutagenesis, or by the PCR method using primers in which desired mutation has been introduced to obtain a DNA fragment in which the desired modification has been introduced. Thereafter, the mutated DNA fragment may be ligated to a DNA fragment encoding another portion of the enzyme of interest.

Alternatively, in order to obtain DNA encoding a protein comprising a shortened amino acid sequence, an amino acid sequence longer than the amino acid sequence of interest, for example, DNA encoding the full-length amino acid sequence, may be cleaved with a desired restriction enzyme, and when the resultant DNA fragment was found not to encode the entire amino acid sequence of interest, a DNA fragment comprising the sequence of the lacking portion may be synthesized and ligated thereto.

The present invention is not limited to a gene encoding a protein that has an activity of regulating the pH of vacuoles derived from morning glory, but the sources may be plants, animals, or microorganisms, and all they need is to have a topology that pumps protons out of the vacuole.

By expressing the obtained gene using a gene expression system in Escherichia coli or yeast and determining the activity, it can be confirmed that the gene obtained encodes a protein that has an activity of regulating the pH of vacuoles. Furthermore, by expressing said gene, a protein, the gene product, having an activity of regulating the pH of vacuoles can be

obtained. Alternatively, a protein can also be obtained that has an activity of regulating the pH of vacuoles using an antibody against the amino acid sequence as set forth in SEQ ID NO: 2, and a protein that has an activity of regulating the pH of vacuoles derived from other organisms can be cloned using an antibody.

Thus, the present invention also relates to a recombinant vector comprising the above-mentioned gene, specifically an expression vector, and a host cell transformed with said vector. As a host, there can be used a prokaryotic or eukaryotic organism. As a prokaryotic organism, for example, there can be used such a common host as a bacterium belonging to the genus Escherichia such as Escherichia coli, a bacterium belonging to the genus Bacillus such as Bacillus subtilis, and the like. As a eukaryotic host, there can be used a lower eukaryotic organism, for example an eukaryotic microorganism such as a fungus, a yeast or a mold.

As yeast, there can be mentioned a microorganism belonging to the genus Saccharomyces such as Saccharomyces cerevisiae, and as a mold, there can be mentioned a microorganism belonging to the genus Aspergillus such as Aspergillus oryzae and Aspergillus niger, and a microorganism belonging to the genus Penicillium. Furthermore, animal cells or plant cells can be used: as animal cells, there can be used cell lines derived from mouse, hamster, monkey, human and the like. Insect cells such as silkworm cells or adult silkworms per se can also be used as hosts.

The vectors of the present invention may contain expression regulatory regions such as a promoter, a terminator, an origin of replication, and the like, depending on the type of the host into which said vector is to be introduced. As promoters for bacterial expression vectors, there can be used commonly used promoters such as trc promoter, tac promoter, lac

promoter, and the like; as promoters for yeasts, there can be used the glyceraldehyde-3-phosphate dehydrogenase promoter, PHO5 promoter, and the like; and as mold promoters, there can be used amylase promoter, trpC promoter, and the like.

As promoters for animal cell hosts, there can be used viral promoters such as SV40 early promoter, SV40 late promoter, and the like. The construction of expression vectors may be performed according to conventional methods using restriction enzymes, ligase, etc. The transformation of host cells can also be performed according to conventional methods.

Host cells transformed with the above expression vectors may be cultured, cultivated or bred, and from the culture the desired protein can be recovered and purified according to conventional methods such as filtration, centrifugation, cell disruption, gel filtration chromatography, ion exchange chromatography, and the like.

The present invention also relates to a plant or its progenies or tissues thereof of which hue of color has been controlled by introducing a gene encoding a protein that has an activity of regulating the pH of the vacuoles, specifically a Na^+-H^+ antiporter gene. They may be cut flowers in shape. Using a gene encoding a protein that has an activity of regulating the pH of vacuoles obtained by the present invention, the pumping of proton into the cytoplasm from the vacuole and the pumping of sodium ion into the vacuole can be performed, so that anthocyanins accumulated in the vacuole can be turned blue and, as a result, the flower color can be turned blue.

It is also possible to lower the pH of vacuoles by suppressing the expression of the gene of the present invention. With the state-of-the-art technology, it is possible to introduce a gene into plants, and allow the gene to be expressed in a constitutive or tissue-specific

manner, and also to suppress the expression of the gene of interest by the antisense method or the co-suppression method.

Examples of plants that can be transformed include, but not limited to, roses, chrysanthemums, carnations, snapdragons, cyclamens, orchids, lisianthus, freesias, gerberas, gladioluses, gypsophilas, kalanchoes, lilies, pelargoniumas, geraniums, petunias, torenias, tulips, rice, barley, whieat, rapeseeds, potatoes, tomatoes, poplars, bananas, eucalyptuses, sweet potatoes, soy beans, alfalfas, lupins, corns, and the like.

Examples

The present invention will now be explained in further details with reference to the following Examples. Molecular biological techniques used were performed according to Molecular Cloning (Sambrook et al., 1989), unless otherwise specified.

Example 1. Obtaining a germ cell revertant

Obtaining a germ cell revertant has already been reported (Iida et al., Shokubutsu Saibo Kogaku Series (Plant Cell Engineering Series) 5 (1996) pp. 132, Shujunsha; Iida et al., Annal. New York Acad. Sci. (1999) pp. 870; Inagaki et al., Plant Cell, 6:375 (1994); Inagaki et al., Theor. Appl. Genet. 92:499 (1996)).

Morning glory having the genotype (Pr-r/pr-m) (Iida et al., pp. 870; Inagaki et al., Plant Cell, 6:375 (1994); Inagaki et al., Theor. Appl. Genet. 92:499 (1996)) was subjected to self-fertilization and the seeds of the progeny were planted. The flowers of the self-fertilized progeny were observed to select individuals that bloom with blue flowers by back mutation. Furthermore, in this self-fertilized progeny of the germ cell revertant, it was proved whether it is homozygous or heterozygous based on whether or not isolates that bloom with purple flowers can be obtained. Those having the genotype (Pr-r/Pr-r) and (pr-m/pr-m) were selected.

Example 2. Anthocyanins in the petals of revertants

Anthocyanins contained in morning glory are mainly heavenly blue anthocyanin and several other anthocyanins (Lu et al., Phytochemistry 31:659 (1992)). When the open petals of the Pr-r/Pr-r strain and the pr-m/pr-m strain obtained in Example 1 were similarly analyzed, the anthocyanins contained in both of them were almost identical.

A cellophane tape was stuck to the front side of a petal and then peeled off to recover one layer of epithelium, from which the cell liquid was scraped with a scalpel etc., which was then centrifuged to obtain juice. The pH of the juice was measured using the Horiba B212 pH meter (Horiba Seisakusho). pH of the petal epithelium of the Pr-r/Pr-r strain was about 7.1 whereas that of the pr-m/pr-m strain was about 6.5. This result indicates that the change in flower color by mutation of purple was not due to the structure of anthocyanins but to the change of vacuolar pH.

Example 3. Isolation of a genome fragment specifically present in pr-m

For the isolation of a gene, the transposon display method (Frey et al., Plant J. 13:717 (1998); Van den Broeck et al., Plant J. 13:121 (1998)) or a similar method (Dosho et al., Shokubutsu Saibo Kogaku Series (Plant Cell Engineering Series) 7 (1997) pp. 144, Shujunsha) was used to search for DNA bands that were present in the pr-m/pr-m strain and the Pr-w/pr-m strain but not in the Pr-r/Pr-r strain or in the wild strain. Since Tpn1-related transposon is thought to be mainly associated with mutability in morning glory, special note was given to the Tpn1-related transposon.

Specifically, chromosomal DNA was extracted from the pr-m/pr-m strain, and 125 ng of it was digested with MseI in 20 μl. To the digested DNA was added 80 pmole of MseI adaptor (obtained by annealing 5'-GACGATGAGTCCTGAG-3' (SEQ ID NO: 3) and 5'-TACTCAGGACTCAT-3' (SEQ ID NO: 4))

in 25 μl at 20°C for 2 hours. After keeping it at 75°C for 10 minutes, it was stored at -20°C. After diluting this ten-fold, 2 μl was used as a template, which was PCR-amplified using 4.8 pmole of TIR primer (5'-TGTGCATTTTTCTTGTAGTG-3' (SEQ ID NO: 5), this includes the inverted terminal repeat of the transposon Tpn1) and 4.8 pmole of MseI primer (5'-GATGAGTCCTGAGTAA-3') (SEQ ID NO: 6) in 20 μl.

PCR was performed with Taq polymerase (Takara Shuzo) for 20 cycles with one cycle comprising 94°C for 0.5 minute, 56°C for 1 minute, and 72°C for 1 minute, and the volume was diluted ten-fold. Two μl of it was used as a template in a PCR using 4.8 pmole of TIR+N primer (5'-TGTGCATTTTTCTTGTAGN-3' (SEQ ID NO: 7) N=A, C, G or T. Four different species were synthesized instead of a mixture) and 4.8 pmole of MseI+N primer (5'-GATGAGTCCTGAGTAAN-3' (SEQ ID NO: 8) N=A, C, G or T. Four different species were synthesized instead of a mixture. The 5'-end was labeled with fluorescein (using Amersham Pharmacia Biotek, Vistra fluorescence 5'-oligo labeling kit)) in 20 μl.

Reactions were performed for combinations of primers to a total of 16 reactions. PCR was performed for 13 cycles with one cycle comprising 94°C for 0.5 minute, 65°C (with a decrement of 0.7°C for each cycle) for 1 minute, and 72°C for 1 minute, and further for 13 cycles with one cycle comprising 94°C for 0.5 minute, 56°C for 1 minute, and 72°C for 1 minute. A similar procedure was performed for chromosomal DNA obtained from the Pr-r/Pr-r strain, subjected to electrophoresis using a sequence gel of the DNA Sequencer 377 (PE Biosystems Japan), and the bands were detected using FMBIOII (Takara Shuzo).

When bands derived from the Pr-r/Pr-r strain and the pr-m/pr-m strain were compared, an about 130 bp DNA fragment was specifically expressed in the strain having

pr-m. The 130 bp DNA fragment was recovered, and amplified by PCR (for 30 cycles with one cycle comprising 94°C for 0.5 minute, 56°C for 1 minute, and 72°C for 1 minute) using 20 pmole TIR primer and 20 pmole MseI primer, which was then subcloned into the pGEM-T vector (Promega Corporation), and then the nucleotide sequence was determined. The sequence was

5'-TGAGCATTTTTCTTGTAGTG CTGAGATTTTCCTCCATTTGTCTGAAGCTCTTCATCCTTCAACAC TACCCCCACATCTCACCTTTCAAG GTCCAATCTTTATCATTCATCT TTACTCAGGACTCATCGTC-3'

(SEQ ID NO: 9) (the single-underlined portion corresponds to a used primer, the double-underlined portion corresponds to an exon, and the rest corresponds to an intron). After the sequence as set forth in SEQ ID NO: 9 was used as a probe in Northern analysis, a transcription product of about 2.3 kb was found in the bud of morning glory having Pr-r, but a corresponding transcription product was not found in the pr-m/pr-m strain. Thus, it can be seen that this 2.3 kb transcription product corresponds to the Purple gene.

Example 4. Isolation of cDNA

About 6 million clones of a cDNA library (Inagaki et al., Plant Cell 6:375 (1994)) derived from the wild strain morning glory (Pr-w/Pr-w) were screened using the 130 bp DNA fragment as a probe, with a result that two positive clones were obtained. One of these clones had a 2237 bp cDNA, among which a 1626 bp-long open reading frame was observed (SEQ ID NO: 1). The predicted amino acid sequence had an identity of 29.3% and 73.4% with the Na^+-H^+ antiporter of yeast and Arabidopsis, respectively (Nhx1 and AtNhx1, respectively, Gaxiola et al., Proc. Natl. Acad. Sci. USA 96:1480-1485 (1999)).

The result revealed that the Purple gene of morning glory encodes a Na^+-H^+ antiporter. Incidentally, although the Na^+-H^+ antiporter obtained from Arabidopsis is attracting attention as a protein that gives salt resistance to yeast, this is the first time that an association of the Na^+-H^+ antiporter with flower color

was observed.

Example 5. Complementation experiment of yeast Na^+-H^+ antiporter

The predicted amino acid sequence encoded by the Purple gene of morning glory has a homology with those of the Na^+-H^+ antiporters of yeast and Arabidopsis. Thus, in order to confirm whether the Purple gene product of morning glory can function as a Na^+-H^+ antiporter protein, a complementation experiment was performed using a yeast Na^+-H^+ antiporter mutant.

First, the following two DNA fragments were synthesized:

CBSCl-Linker (22 mer) 5'-CGA TAG ATC TGG GGG TCG ACA T-3' (SEQ ID NO: 12)

CSBD2-Linker (22 mer) 5'-CGA TGT CGA CCC CCA GAT CTA T-3' (SEQ ID NO: 13)

From these two fragments, a linker having restriction enzyme sites ClaI-BglII-SalI-ClaI is formed. A plasmid pINA145 (Fig. 3) was constructed by inserting the above linker according to a standard method into the ClaI site of the pYES2 vector (Invitrogen Corporation) so that the BglII site is located at the URA3 gene side. A plasmid pINA147 (Fig. 4) was constructed by ligating a 2 kb DNA fragment obtained by digesting plasmid pJJ250 (Jones and Prakash, Yeast 6:363-366 (1990)) with BamHI and SalI to plasmid pINA145 digested with BglII and SalI. Plasmid pIAN151 was constructed by ligating Purple cDNA thereto under the control of the GAL 1 promoter of plasmid pINA147. pINA147 and pIAN151 were transformed respectively to the yeast R101 strain which is a mutant strain of the Na^+-H^+ antiporter. Due to the mutation of the Na^+-H^+ antiporter, the yeast R101 strain cannot grow on a 400 mM NaCl-added APG medium (Nass et al., J. Biol. Chem. 272:26145 (1997); Gaxiola et al., 96:1480-1485 (1999)). The pINA147-transformed R101 strain could not grow either, and only the pIAN151-transformed R101 strain could grow on the 400 mM NaCl-added APG medium. The

result has shown that the gene product of the morning glory Purple gene has the Na^+-H^+ antiporter function.

Example 6. Construction of an expression vector in plants

With 10 ng of morning glory Purple cDNA as template, PCR was performed using synthetic primers PR-5 (5'-GGGATCCAACAAAAATGGCTGTCGGG-3') (SEQ ID NO: 10) and PR-3 (5'-GGGTCGACTAAGCATCAAAACATAGAGCC-3') (SEQ ID NO: 11). The polymerase used was Taq polymerase (Toyoboseki), and the reaction was performed, after reaction at 95°C for 45 seconds, for 25 cycles with one cycle comprising 95°C for 45 seconds, 50°C for 45 seconds, and 72°C for 45 seconds, and then further reacted at 72°C for 10 minutes. An about 1.6 kb DNA fragment obtained was ligated to pCR2.1-Topo (Clontech) to make pCR-purple. It was confirmed that there were no errors due to PCR in the nucleotide sequence of Purple cDNA on this plasmid.

pBE2113-GUS (Mitsuhara et al., Plant Cell Physiol. 37:49 (1996)) was digested with SacI and blunt-ended. Then a XhoI linker (Toyoboseki) was inserted thereto, and the plasmid obtained was termed pBE2113-GUSx. This was digested with EcoRI and HindIII to obtain an about 2.7 kb DNA fragment, which was ligated to the HindIII and EcoRI digest of pBinPLUS, and the plasmid obtained was termed pBEXP.

On the other hand, an about 1.2 kb DNA fragment obtained by digesting pCGP484 (Kohyo (National Publication of Translated Version) No. 8-511683) with HindIII and XbaI, an about 1.6 kb DNA fragment obtained by digesting pCR-purple with XbaI and SalI, and an about 13 kb DNA fragment obtained by digesting pBEXP with HindIII and XhoI were ligated to obtain pSPB607 (Fig. 1). This plasmid is a binary vector for use in the Agrobacterium-mediated transformation of plants, and on this plasmid Purple cDNA is under the control of a chalcone synthase promoter derived from snapdragon and a nopaline synthase terminator derived from Agrobacterium.

An about 0.8 kb DNA fragment obtained by digesting pCGP669 (Kohyo (National Publication of Translated Version) No. 8-511683) with HindIII and BamHI, an about 1.6 kb DNA fragment obtained by digesting pCR-purple with BamHI and SalI, and an about 13 kb DNA fragment obtained by digesting pBEXP with HindIII and XhoI were ligated to obtain pSPB608 (Fig. 2). This plasmid is a binary vector for use in the Agrobacterium-mediated transformation of plants, and on this plasmid Purple cDNA is under the control of a chalcone synthase promoter derived from petunia and a nopaline synthase terminator derived from Agrobacterium.

By transforming plants using the expression vectors thus obtained, the pH of vacuoles can be regulated and thereby flower color can be controlled.

Example 7. Isolation of a homologs of the Purple gene

cDNA libraries derived from the petals of petunia (Petunia hybrida cv. Old Glory Blue), Nierembergia (Nierembergia hybrida cv. NB17), and Torenia (Torenia hybrida cv. Summerwave Blue) were each constructed using the cDNA synthesis kit (Stratagene, USA). The method of construction was as recommended by the manufacturer. About 200,000 clones each were screened according to a standard method. For washing the membrane, an aqueous solution of 5 × SSC and 0.1% SDS was used and the incubation was performed three times at 50°C for 10 minutes. Among the positive clones obtained, the nucleotide sequence of the longest clone was determined for each clone. The nucleotide sequence of the clone of Petunia and the corresponding amino acid sequence are shown in SEQ ID NO: 14 and 15, the nucleotide sequence of the clone of Nierembergia and the corresponding amino acid sequence are shown in SEQ ID NO: 16 and 17, and the nucleotide sequence of the clone of Torenia and the corresponding amino acid sequence are shown in SEQ ID NO: 18 and 19. Homologs of the Purple gene of Petunia, Nierembergia, and Torenia had an identity on the amino

acid level of 75%, 76%, and 71%, respectively, with the morning glory Purple gene.

Since the amino acid sequence of the Na^+-H^+ antiporter encoded by the morning glory Purple gene and that of the Na^+-H^+ antiporter encoded by Arabidopsis AtNhx 1 are about 73% identical, the homologs of the Purple gene of Petunia, Nierembergia, and Torenia obtained are judged to encode the Na^+-H^+ antiporter.

Example 8. Isolation of the clone of morning glory Purple chromosome

After chromosomal DNAs of a mutant morning glory (pr-m/pr-m) and a revertant morning glory (Pr-r/Pr-r) were cleaved with BglII, they were electrophoresed on a 0.8% agarose gel, and were subjected to genomic Southern analysis with cDNA of morning glory Purple as a probe. As a result, an about 7.5 kb band that was not present in the mutant morning glory was detected in the revertant morning glory.

After 50 μg of chromosomal DNA of the wild type morning glory (Pr-w/Pr-w, the KKZSK2 strain) was digested with BglII, it was electrophoresed on a 0.8% agarose gel. An about 7-9 kb fragmently was recovered, from which DNA was extracted using the GENECLEAN III KIT (B10101). This DNA was ligated to the λ Zap express vector (Stratagene, USA), which was screened with cDNA of morning glory Purple as a probe. The determination of nucleotide sequences of positive clones obtained revealed that, on this about 7.5 kb DNA fragment, there was a region from about 6.3 kb upstream of the Purple promoter to midway in exon 3. For this sequence, a sequence up to the initiation codon of the Purple gene is shown in SEQ ID NO: 20.

It has been demonstrated that the expression of the Purple gene is strongly induced only at about 24 hours before the flowering of morning glory, and that the expression of the Purple gene is suppressed by insertion

of a transposon into the 5'-untranslated region. From this, it is clear that the promoter region of the Purple gene obtained contains a factor needed for the expression of the Purple gene in a developmental stage-specific and organ-specific manner in the petals of morning glory. By placing the gene of interest downstream of this promoter region, the expression of the gene of interest can be regulated in a developmental stage-specific and organ-specific manner.

Industrial Applicability

The gene obtained in the present invention was found, for the first time, to be involved in controlling the pH of vacuoles and flower color. By expressing the gene of the present invention on the flower petals, the pH of vacuoles can be increased and thereby the flower color can be turned blue. Furthermore, by suppressing the expression of the gene of the present invention, the pH of vacuoles can be lowered and thereby flower color can be turned red. As the gene encoding a protein that regulates the pH of vacuoles, there can be used not only those derived from morning glory obtained in the present invention but also similar genes derived from other organisms.

CLAIMS

1. A gene encoding a protein that has an activity of regulating the pH of vacuoles in plant cells.

2. A gene encoding a protein that has the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles in plant cells.

3. A gene encoding a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other amino acids in the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles.

4. The gene according to claim 1 encoding a protein that has an amino acid sequence having a identity of 20% or more with the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles.

5. The gene according to claim 1 encoding a protein that has an amino acid sequence having a identity of 70% or more with the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles.

6. The gene according to claim 1 that hybridizes to a part or all of a nucleic acid having a nucleotide sequence encoding the amino acid sequence as set forth in SEQ ID NO: 2 under a stringent condition, and that encodes a protein having an activity of regulating the pH of vacuoles.

7. A vector comprising the gene according to any one of the claims 1 to 6.

8. A host cell transformed with the vector according to claim 7.

9. A protein encoded by the gene according to any one of the claims 1 to 6.

10. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method

comprising culturing or growing the host cell according to claim 8 and then harvesting said protein from said host cell.

11. A plant in which the gene according to any one of the claims 1 to 6 or the vector according to claim 7 has been introduced or an progeny thereof having the same property as said plant, or a tissue thereof.

12. A cut flower of the plant according to claim 11 or an progeny thereof having the same property as said plant.

13. A method of regulating the pH of vacuoles comprising introducing the gene according to any one of the claims 1 to 6 or the vector according to claim 7 into a plant or plant cells and then allowing said gene to be expressed.

14. A method of controlling the flower color of plants comprising introducing the gene according to any one of the claims 1 to 6 or the vector according to claim 7 into a plant or plant cells and then allowing said gene to be expressed.

Fig.1

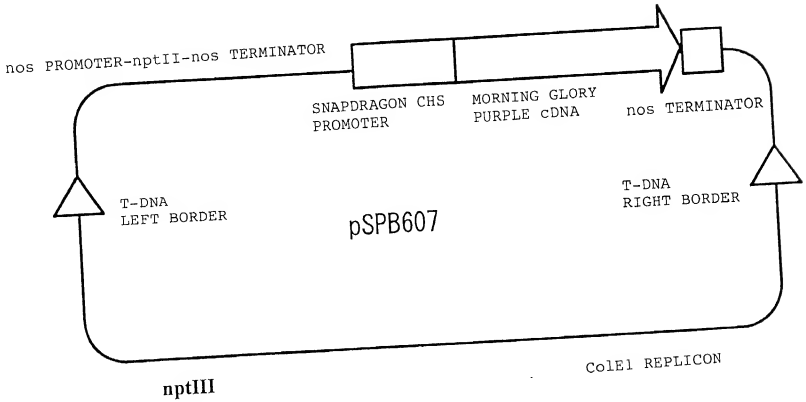


Fig. 2

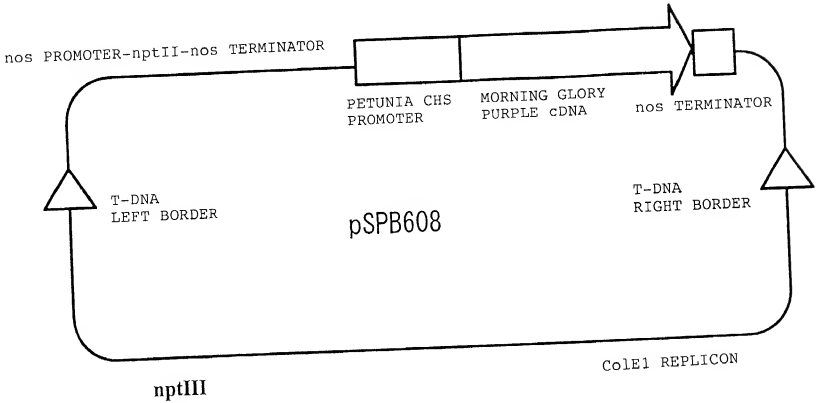


Fig.3

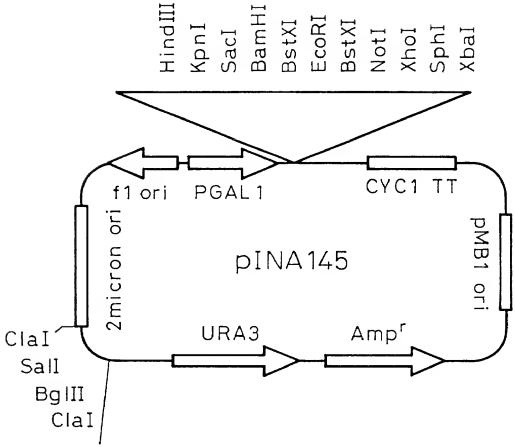
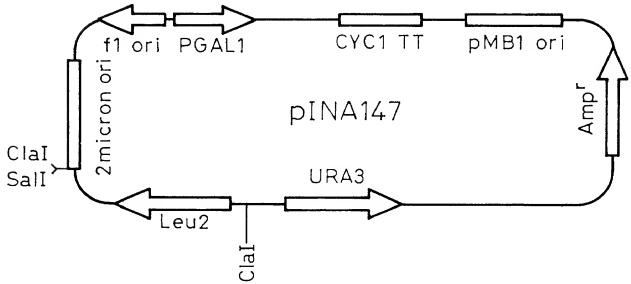

HindIII
KpnI
SacI
BamHI
BstXI
EcoRI
BstXI
NotI
XhoI
SphI
XbaI
f1 ori
PGAL1
CYC1 TT
2micron ori
pINA145
pMB1 ori
ClaI
SalI
BglII
ClaI
URA3
Ampr

Fig.4


f1 ori
PGAL1
CYC1 TT
pMB1 ori
2micron ori
pINA147
Ampr
ClaI
SalI
URA3
Leu2
ClaI

PTO/SB/105 (6-95)
Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Declaration and Power of Attorney For Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者であると（下記の名称が複数の場合）信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

GENES ENCODING PROTEINS REGULATING

THE pH OF VACUOLES

上記発明の明細書（下記の欄でx印がついていない場合は、本書に添付）は、

the specification of which is attached hereto unless the following box is checked:

☐ __月__日に提出され、米国出願番号または特許協定条約国際出願番号を_____とし、
（該当する場合）_____ に訂正されました。

☐ was filed on August 24, 2000
as United States Application Number or
PCT International Application Number
PCT/JP00/05722 and was amended on
_____ (if applicable).

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第３７編第１条５６項に定義されるとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

Japanese Language Declaration
(日本語宣言書)

私は、米国法典第３５編１１９条(a)-(d)項又は３６５条(b)項に基き下記の、米国以外の国の少なくとも一ヵ国を指定している特許協力条約３６５条(a)項に基づく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

I hereby claim foreign priority under Title 35, United States Code, Section 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)
外国での先行出願

(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	Priority Not Claimed 優先権主張なし
11-236800(Pat.Appln.)	Japan	24/August/1999	☐
			☐

私は、第３５編米国法典１１９条(e)項に基いて下記の米国特許出願規定に記載された権利をここに主張いたします。

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Application No.) (出願番号)	(Filing Date) (出願日)

私は、下記の米国法典第３５編１２０条に基いて下記の米国特許出願に記載された権利、又は米国を指定している特許協力条約３６５条(c)に基づく権利をここに主張します。また、本出願の各請求範囲の内容が米国法典第３５編１１２条第１項又は特許協力条約で規定された方法で先行する米国特許出願に開示されていない限り、その先行米国出願書提出日と共同で本出願書の日本国内または特許協力条約国際提出日までの期間中に入手された、連邦規則法典第３７編１条５６項で定義された特許資格の有無に関する重要な情報について開示義務があることを認識しています。

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of application.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済)

(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済)

私は、私自身の知識に基づいて本宣言書中で私が行なう表明が真実であり、かつ私の入手した情報と私の信じるところに基づく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第１８編第１００１条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Japanese Language Declaration
(日本語宣言書)

委任状： 私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。(弁護士、または代理人の氏名及び登録番号を明記のこと)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith *(list name and registration number)*

William L. Mathis	17,337	Robert G. Mukai	28,531	William H. Benz	25,952
Peter H. Smolka	15,913	George A. Hovanec, Jr.	28,223	Peter K. Skiff	31,917
Robert S. Swecker	19,885	James A. LaBarre	28,632	Richard J. McGrath	29,195
Platon N. Mandros	22,124	E. Joseph Gess	28,510	Matthew L. Schneider	32,814
Benton S. Duffett, Jr.	22,030	R. Danny Huntington	27,903	Michael G. Savage	32,596
Joseph R. Magnone	24,239	Eric H. Weisblatt	30,505	Gerald F. Swiss	30,113
Norman H. Stepno	22,716	James W. Peterson	26,057	Michael J. Ure	33,089
Ronald L. Grudziecki	24,970	Teresa Stanek Rea	30,427	Charles F. Wieland III	33,096
Frederick G. Michaud, Jr.	26,003	Robert E. Krebs	25,885	Bruce T. Wieder	33,815
Alan E. Kopecki	25,813	William C. Rowland	30,888	Todd R. Walters	34,040
Regis E. Slutter	26,999	T. Gene Dillahunty	25,423		
Samuel C. Miller, III	27,360	Patrick C. Keane	32,858		
Ralph L. Freeland, Jr.	16,110	Bruce J. Boggs, Jr.	32,344		

書類送付先

Send Correspondence to:
Ronald L. Grudziecki
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404

直接電話連絡先： (名前及び電話番号)

Direct Telephone Calls to: *(name and telephone number)*
Ronald L. Grudziecki
at (703) 836-6620

第一または第一発明者名		Full name of sole or first inventor Shigeru Iida
発明者の署名	日付	Inventor's signature Date April 16, 2001
住所		Residence Okazaki-shi, Aichi, Japan JPX
国籍		Citizenship Japanese
私書箱		Post Office Address 2-4-1-3-21, Tatsumi-minami, Okazaki-shi, Aichi 444-0874, Japan
第二共同発明者		Full name of second joint inventor, if any Sachiko Tanaka
第二共同発明者	日付	Second inventor's signature Date April 16, 2001
住所		Residence Okazaki-shi, Aichi, Japan JPX ST
国籍		Citizenship Japanese
私書箱		Post Office Address 61, Gohonmatsu, Miai-cho, Okazaki-shi, Aichi 444-0802, Japan ST

(第三以降の共同発明者についても同様に記載し、署名をすること)

(Supply similar information and signature for third and subsequent joint inventors.)

第三共同発明者		Full name of third joint inventor, if any Yoshishige Inagaki
第三共同発明者	日付	Third inventor's signature Date April 16, 2001
住　所		Residence Okazaki-shi, Aichi Japan
国　籍		Citizenship Japanese
私書箱		Post Office Address Jonanhaitsu 105, 2, Miyashita, Kugosaki-cho, Okazaki-shi, Aichi 444-0851, Japan
第四共同発明者		Full name of fourth joint inventor, if any
第四共同発明者	日付	Fourth inventor's signature Date
住　所		Residence
国　籍		Citizenship
私書箱		Post Office Address

第五共同発明者		Full name of fifth joint inventor, if any
第五共同発明者	日付	Fifth inventor's signature Date
住　所		Residence
国　籍		Citizenship
私書箱		Post Office Address
第六共同発明者		Full name of sixth joint inventor, if any
第六共同発明者	日付	Sixth inventor's signature Date
住　所		Residence
国　籍		Citizenship
私書箱		Post Office Address

（第七以降の共同発明者についても同様に記載し、署名をすること）

(Supply similar information and signature for seventh and subsequent joint inventors.)

SEQUENCE LISTING

<110> SUNTORY LIMITED

<120> Gene encoding for proteins regulating the pH of vacuoles

<130> 994020

<160> 20

<210> 1
<211> 2237
<212> DNA
<213> Ipomea nil

<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

<400> 1

```
agaatgtagg ctacagaaat tttcagacag atagatacat aaatccgtat aatagagaca   60
gagaaacaga aaaagagaga gtcacgttaa tcctgagatt ttcctccatt tgtctgaagc  120
tcttcatcct tcaacactac ccccacatct caccttttcaa gtgatttgta tgttttcggg  180
agggattgga atgggcaacc cggatatgtg aacagaaacc acgacattgg gaaaagattt  240
attgcaaaaa ttgttttgat tgttttggat tttgtggtag aaaaagggga agaacaaaa   299
atg gcg ttc ggg ttg tct tct ttg ctc caa aat tcg gat ttg ttc acg     347
Met Ala Phe Gly Leu Ser Ser Leu Leu Gln Asn Ser Asp Leu Phe Thr
  1               5                  10                  15
tct gat cat gct tcc gtt gtg tcg atg aac ctc ttt gtg gcg ttg ctt    395
Ser Asp His Ala Ser Val Val Ser Met Asn Leu Phe Val Ala Leu Leu
            20                  25                  30
tgc gca tgc att gtt ctt ggc cat cta ctc gag gag aat cgc tgg gtg    443
Cys Ala Cys Ile Val Leu Gly His Leu Leu Glu Glu Asn Arg Trp Val
        35                  40                  45
```

```
aac gaa tcc att act gcc ctt ata att ggt ttg tgc acc gga gtt gta    491
Asn Glu Ser Ile Thr Ala Leu Ile Ile Gly Leu Cys Thr Gly Val Val
     50                  55                  60

att ttg ctc ctt agc gga gga aag agt tca cat ctt ctc gtc ttt agc    539
Ile Leu Leu Leu Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser
 65                  70                  75                  80

gaa gat ctt ttc ttt ata tat ctc ctg cca cct ata ata ttc aat gcg    587
Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala
                 85                  90                  95

ggg ttt caa gtg aaa aag aag cag ttt ttc gtg aac ttc atg aca att    635
Gly Phe Gln Val Lys Lys Lys Gln Phe Phe Val Asn Phe Met Thr Ile
            100                 105                 110

atg ctg ttt gga gct att ggc aca ctt att agc tgt tct att ata tca    683
Met Leu Phe Gly Ala Ile Gly Thr Leu Ile Ser Cys Ser Ile Ile Ser
        115                 120                 125

ttt ggt gcg gtc aaa att ttc aag cac tta gac att gac ttt ctg gat    731
Phe Gly Ala Val Lys Ile Phe Lys His Leu Asp Ile Asp Phe Leu Asp
    130                 135                 140

ttt gga gat tat tta gca att ggt gcg ata ttt gct gca acc gat tct    779
Phe Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser
145                 150                 155                 160

gtt tgc aca ttg cag gtg ctc agt cag gat gag acg ccc cta ctt tac    827
Val Cys Thr Leu Gln Val Leu Ser Gln Asp Glu Thr Pro Leu Leu Tyr
                165                 170                 175

agt ctc gtg ttt gga gaa ggg gtc gtc aat gat gct aca tct gtg gtc    875
Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val
            180                 185                 190

ctt ttt aat gct att caa agt ttt gac atg act agt ttt gat cca aaa    923
Leu Phe Asn Ala Ile Gln Ser Phe Asp Met Thr Ser Phe Asp Pro Lys
        195                 200                 205

att ggg ctt cat ttc att gga aac ttc ttg tat tta ttt ctc tcg agc    971
Ile Gly Leu His Phe Ile Gly Asn Phe Leu Tyr Leu Phe Leu Ser Ser
    210                 215                 220

act ttt ttg ggc gtg gga att gga ctg ctt tgt gct tat att atc aaa   1019
Thr Phe Leu Gly Val Gly Ile Gly Leu Leu Cys Ala Tyr Ile Ile Lys
225                 230                 235                 240
```

```
aag cta tac ttt ggc agg cac tca acc gat cgt gag gtt gcc ctt atg   1067
Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Leu Met
                245                 250                 255
atg ctc atg tct tac ttg tct tat ata atg gcc gag tta ttc tat cta   1115
Met Leu Met Ser Tyr Leu Ser Tyr Ile Met Ala Glu Leu Phe Tyr Leu
            260                 265                 270
agc ggc ata ctt act gta ttc ttc tgt gga att gtc atg tct cat tat   1163
Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr
        275                 280                 285
acc tgg cac aat gtt acc gag agc tca agg gtc act act agg cat tcc   1211
Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Arg His Ser
    290                 295                 300
ttt gca act ctg tca ttt gtc gca gag aca ttt atc ttc ctc tat gtt   1259
Phe Ala Thr Leu Ser Phe Val Ala Glu Thr Phe Ile Phe Leu Tyr Val
305                 310                 315                 320
ggt atg gat gcc ttg gat atc gag aaa tgg aaa ttt gtg aaa aat agt   1307
Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Lys Asn Ser
                325                 330                 335
cag gga cta tca gtt gca gtg agc tca ata ttg gta ggc cta atc tta   1355
Gln Gly Leu Ser Val Ala Val Ser Ser Ile Leu Val Gly Leu Ile Leu
            340                 345                 350
gta ggc aga gct gcg ttc gta ttc ccc ttg tcg ttt tta tcc aac tta   1403
Val Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu
        355                 360                 365
gca aag aaa aac tct tcg gac aag ata tcc ttt agg caa caa ata ata   1451
Ala Lys Lys Asn Ser Ser Asp Lys Ile Ser Phe Arg Gln Gln Ile Ile
    370                 375                 380
att tgg tgg gct ggc cta atg aga ggc gcc gtc tca ata gca ctt gcg   1499
Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Ile Ala Leu Ala
385                 390                 395                 400
tat aat aag ttt aca acc tcg ggg cat acg tca ttg cac gag aac gca   1547
Tyr Asn Lys Phe Thr Thr Ser Gly His Thr Ser Leu His Glu Asn Ala
                405                 410                 415
ata atg att aca agt act gtt acg gtt gtt ctg ttc agc aca gtt gta   1595
Ile Met Ile Thr Ser Thr Val Thr Val Val Leu Phe Ser Thr Val Val
            420                 425                 430
```

```
ttc ggg ttg atg acg aag cct ctg ata aac ctt ctg cta ccc ccg cac   1643
Phe Gly Leu Met Thr Lys Pro Leu Ile Asn Leu Leu Leu Pro Pro His
        435                 440                 445
aag cag atg cca agc ggt cat tcg tca atg aca aca tcc gaa ccc agt   1691
Lys Gln Met Pro Ser Gly His Ser Ser Met Thr Thr Ser Glu Pro Ser
    450                 455                 460
agt ccg aag cac ttc acg gtg cca ctc ctg gac aac caa cct gac tca   1739
Ser Pro Lys His Phe Thr Val Pro Leu Leu Asp Asn Gln Pro Asp Ser
465                 470                 475                 480
gaa agc gat atg ata acc gga cct gag gtt gct cga cca act gcc ttg   1787
Glu Ser Asp Met Ile Thr Gly Pro Glu Val Ala Arg Pro Thr Ala Leu
                485                 490                 495
cgc atg ctg cta agg acg cca acc cac acc gtg cac cgc tac tgg cgt   1835
Arg Met Leu Leu Arg Thr Pro Thr His Thr Val His Arg Tyr Trp Arg
            500                 505                 510
aag ttt gat gat tcg ttt atg cgt ccc gtg ttt ggc ggg cgg gga ttc   1883
Lys Phe Asp Asp Ser Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe
        515                 520                 525
gtt ccg ttt gtc gcg ggc tca cca gtt gag cag agc cct aga tga       1928
Val Pro Phe Val Ala Gly Ser Pro Val Glu Gln Ser Pro Arg
    530                 535                 540
ggtacaaagt acaaacaaga cactgttgct gggtgaaata gtgtaagttg tatcatagtt 1988
gattctggtt gcccctctta tgaaatgggc tgggtgaaag tcttctcact agctaggttg 2048
cattgcattg ctacttcata aatgttttat tttattttgt aaatgttggt gcattttagg 2108
tacttgtatt aacacctcat ttgtagcata ttatttggta cagagtattt tttttatgaa 2168
acaataatgg ctgaattatc aatttggctc tatgttttga tgcttagtaa aaaaaaaaaa 2228
aaaaaaaaa                                                        2237
```

<210> 2
<211> 542
<212> PRT
<213> Ipomea nil

<223> Amino acid sequence of protein regulating the pH of vacuoles

<400> 2

Met Ala Phe Gly Leu Ser Ser Leu Leu Gln Asn Ser Asp Leu Phe Thr
1 5 10 15

Ser Asp His Ala Ser Val Val Ser Met Asn Leu Phe Val Ala Leu Leu
20 25 30

Cys Ala Cys Ile Val Leu Gly His Leu Leu Glu Glu Asn Arg Trp Val
35 40 45

Asn Glu Ser Ile Thr Ala Leu Ile Ile Gly Leu Cys Thr Gly Val Val
50 55 60

Ile Leu Leu Leu Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser
65 70 75 80

Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala
85 90 95

Gly Phe Gln Val Lys Lys Lys Gln Phe Phe Val Asn Phe Met Thr Ile
100 105 110

Met Leu Phe Gly Ala Ile Gly Thr Leu Ile Ser Cys Ser Ile Ile Ser
115 120 125

Phe Gly Ala Val Lys Ile Phe Lys His Leu Asp Ile Asp Phe Leu Asp
130 135 140

Phe Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser
145 150 155 160

Val Cys Thr Leu Gln Val Leu Ser Gln Asp Glu Thr Pro Leu Leu Tyr
165 170 175

Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val
180 185 190

Leu Phe Asn Ala Ile Gln Ser Phe Asp Met Thr Ser Phe Asp Pro Lys
195 200 205

Ile Gly Leu His Phe Ile Gly Asn Phe Leu Tyr Leu Phe Leu Ser Ser
210 215 220

Thr Phe Leu Gly Val Gly Ile Gly Leu Leu Cys Ala Tyr Ile Ile Lys
225 230 235 240

Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Leu Met
245 250 255

Met Leu Met Ser Tyr Leu Ser Tyr Ile Met Ala Glu Leu Phe Tyr Leu
260 265 270

Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr
275 280 285
Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Arg His Ser
290 295 300
Phe Ala Thr Leu Ser Phe Val Ala Glu Thr Phe Ile Phe Leu Tyr Val
305 310 315 320
Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Lys Asn Ser
325 330 335
Gln Gly Leu Ser Val Ala Val Ser Ser Ile Leu Val Gly Leu Ile Leu
340 345 350
Val Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu
355 360 365
Ala Lys Lys Asn Ser Ser Asp Lys Ile Ser Phe Arg Gln Gln Ile Ile
370 375 380
Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Ile Ala Leu Ala
385 390 395 400
Tyr Asn Lys Phe Thr Thr Ser Gly His Thr Ser Leu His Glu Asn Ala
405 410 415
Ile Met Ile Thr Ser Thr Val Thr Val Val Leu Phe Ser Thr Val Val
420 425 430
Phe Gly Leu Met Thr Lys Pro Leu Ile Asn Leu Leu Leu Pro Pro His
435 440 445
Lys Gln Met Pro Ser Gly His Ser Ser Met Thr Thr Ser Glu Pro Ser
450 455 460
Ser Pro Lys His Phe Thr Val Pro Leu Leu Asp Asn Gln Pro Asp Ser
465 470 475 480
Glu Ser Asp Met Ile Thr Gly Pro Glu Val Ala Arg Pro Thr Ala Leu
485 490 495
Arg Met Leu Leu Arg Thr Pro Thr His Thr Val His Arg Tyr Trp Arg
500 505 510
Lys Phe Asp Asp Ser Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe
515 520 525
Val Pro Phe Val Ala Gly Ser Pro Val Glu Gln Ser Pro Arg
530 535 540

<210> 3
<211> 16
<212> DNA
<213> Artificial sequence

<220>
<221>
<222>
<223> MseI adaptor

<400> 3
gacgatgagt cctgag 16

<210> 4
<211> 14
<212> DNA
<213> Artificial sequence

<220>
<221>
<222>
<223> MseI adaptor

<400> 4
tactcaggac tcat 14

<210> 5
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<221>
<222>
<223> TIR primer

<400> 5
tgtgcatttt tcttgtagtg 20

<210> 6
<211> 16
<212> DNA
<213> Artificial sequence

<220>
<221>
<222>
<223> MseI primer

<400> 6
gatgagtcct gagtaa 16

<210> 7
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<221>
<222>
<223> TIR+N primer

<400> 7
tgtgcatttt tcttgtagn 19

<210> 8
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<221>

<222>
<223> MseI+N primer

<400> 8
gatgagtcct gagtaan 17

<210> 9
<211> 130
<212> DNA
<213> Artificial sequence

<220>
<221>
<222>
<223>

<400> 9
ttgagcatttt tcttgtagtg ctgagatttt cctccatttg tctgaagctc ttcatccttc 60
aacactaccc ccacatctca cctttcaagg tccaatcttt atcattcatc tttactcagg 120
actcatcgtc 130

<210> 10
<211> 26
<212> DNA
<213> Artificial sequence

<220>
<221>
<222>
<223> PR-5 primer

<400> 10
gggatccaac aaaaatggct gtcggg 26

<210> 11
<211> 29

<212> DNA
<213> Artificial sequence

<220>
<221>
<222>
<223> PR-3 primer

<400> 11
gggtcgacta agcatcaaaa catagagcc 29

<210> 12
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<221>
<222>
<223> CBSC1-linker

<400> 12
cgatagatct gggggtcgac at 22

<210> 13
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<221>
<222>
<223> CBSC2-linker

<400> 13
cgatgtcgac ccccagatct at 22

<210> 14
<211> 2423
<212> DNA
<213> Petunia hybrida

<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

<400> 14

```
attgcgcttc gtattttact gctgaatgaa atcgtgtttt tttattcagt tcgttgttat   60
taatttcaga gttttttta ttaaaggtgt gtttggttga agaaattgta tttgctgaat   120
tttgcagaag tttttgagtt tttgctaaac tattgtgaga tctgattttg aatttttcca   180
gtggtgtttt aagctcaatt cgacgtcgtt tttactggaa ttctgatcag taaatagggc   240
tattttgatg taaggttgtg aaagtttaca gtttggaagt tgagttagtg aaaaagggga   300
aactttattg tgatattttc acaagtattt ggtgaattca ggttattgag a atg gct   357
                                                          Met Ala
ttt gat ttt ggg acg ttg ttg gga aat gta gac agg tta tcg aca tct    405
Phe Asp Phe Gly Thr Leu Leu Gly Asn Val Asp Arg Leu Ser Thr Ser
  5                  10                  15
gat cat caa tca gtt gtg tcg ata aac tta ttc gtt gct ctt att tgc    453
Asp His Gln Ser Val Val Ser Ile Asn Leu Phe Val Ala Leu Ile Cys
 20                  25                  30
gcg tgt att gtg atc ggt cat ttg ttg gaa gaa aac aga tgg atg aat    501
Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn
 35                  40                  45                  50
gag tcc ata act gcc tta gtg att ggt tct tgt act gga atc gtt att    549
Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Ile Val Ile
                 55                  60                  65
cta ctg ata agt gga gga aag aac tct cat att tta gtg ttc agt gaa    597
Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe Ser Glu
             70                  75                  80
gat ctt ttc ttc att tac ctt ctt ccg cca atc att ttt aat gct ggg    645
Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala Gly
         85                  90                  95
```

```
ttc cag gtg aaa aag aaa tcg ttc ttc cgc aat ttc agc act atc atg    693
Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr Ile Met
    100                 105                 110

ctc ttt ggg gca ctt ggc acc ttg ata tca ttc att att ata tca tta    741
Leu Phe Gly Ala Leu Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Leu
115                 120                 125                 130
ggt gcc att ggc att ttc aag aaa atg aat att gga agc ctt gaa att    789
Gly Ala Ile Gly Ile Phe Lys Lys Met Asn Ile Gly Ser Leu Glu Ile
                135                 140                 145
gga gat tac ctt gca att ggg gca atc ttc tct gct aca gat tct gta    837
Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ser Ala Thr Asp Ser Val
            150                 155                 160
tgc acc tta caa gtg ctt aat cag gat gaa aca ccc tta ttg tac agt    885
Cys Thr Leu Gln Val Leu Asn Gln Asp Glu Thr Pro Leu Leu Tyr Ser
        165                 170                 175
cta gtt ttt ggg gaa ggt gtt gtg aat gat gcc aca tct gta gtt ctg    933
Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu
    180                 185                 190
ttc aat gct atc cag aac ttt gac tta tct cac atc gac acg ggc aaa    981
Phe Asn Ala Ile Gln Asn Phe Asp Leu Ser His Ile Asp Thr Gly Lys
195                 200                 205                 210
gct atg gaa tta gtt gga aac ttt cta tac ttg ttt gcc tca agc act   1029
Ala Met Glu Leu Val Gly Asn Phe Leu Tyr Leu Phe Ala Ser Ser Thr
                215                 220                 225
gcc cta gga gtt gct gct ggc cta ctg agc gcc tat att att aaa aaa   1077
Ala Leu Gly Val Ala Ala Gly Leu Leu Ser Ala Tyr Ile Ile Lys Lys
            230                 235                 240
ctc tac ttt gga agg cac tca act gac cgt gag gtt gct ata atg ata   1125
Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile
        245                 250                 255
ctc atg gct tac cta tct tac atg ctt gct gaa tta ttc tat tta agt   1173
Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr Leu Ser
    260                 265                 270
```

```
gca atc ctc act gtg ttt ttc tct ggg atc gtg atg tct cac tac acc   1221
Ala Ile Leu Thr Val Phe Phe Ser Gly Ile Val Met Ser His Tyr Thr
275                 280                 285                 290
tgg cat aat gtg act gag agc tcg aga gtc act acc aag cac act ttt   1269
Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His Thr Phe
                295                 300                 305
gct aca tta tca ttt att gct gaa ata ttc ata ttc ctt tat gtt ggt   1317
Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly
            310                 315                 320
atg gat gct ttg gac att gag aag tgg aag ttt gta agc gac agc cct   1365
Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp Ser Pro
        325                 330                 335
gga ata tca gtt cag gtt agc tca ata ttg ctg ggt ctt gtt ttg gtt   1413
Gly Ile Ser Val Gln Val Ser Ser Ile Leu Leu Gly Leu Val Leu Val
    340                 345                 350
gga aga gca gca ttt gtt ttc cca ttg tca ttc ttg tcc aac ttg acc   1461
Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Thr
355                 360                 365                 370
aag aaa act cca gag gcg aaa att agt ttt aac cag cag gtt aca ata   1509
Lys Lys Thr Pro Glu Ala Lys Ile Ser Phe Asn Gln Gln Val Thr Ile
                375                 380                 385
tgg tgg gct gga ctt atg aga ggt gcc gtt tct atg gcc ctt gct tat   1557
Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr
            390                 395                 400
aat cag ttt acc agg gga ggt cat act cag tta cgc gca aat gca ata   1605
Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn Ala Ile
        405                 410                 415
atg atc aca agt act atc act gtt gtc ctt ttc agc aca gtc gtg ttt   1653
Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val Val Phe
    420                 425                 430
ggg ttg atg aca aaa cct ttg att aga ata ttg cta ccc tca cac aaa   1701
Gly Leu Met Thr Lys Pro Leu Ile Arg Ile Leu Leu Pro Ser His Lys
435                 440                 445                 450
cac ttg agc aga atg atc tct tct gaa cca acg acc cca aaa tcc ttc   1749
His Leu Ser Arg Met Ile Ser Ser Glu Pro Thr Thr Pro Lys Ser Phe
                455                 460                 465
```

```
att gtg cca ctt ctt gac agc aca caa gac tca gaa gct gat ctg gaa   1797
Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp Leu Glu
            470                 475                 480
cgc cat gta ccc cgt ccc cac agt ttg cgg atg ctc ctt tca acc cca   1845
Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser Thr Pro
        485                 490                 495
tct cat aca gtg cat tat tac tgg aga aag ttt gac aat gca ttc atg   1893
Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met
    500             505                 510
cgt cca gtt ttc ggt gga cga ggt ttt gta cct ttt gct cca gga tca   1941
Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Ala Pro Gly Ser
515                 520                 525                 530
ccg aca gac cca gtt ggt gga aat ttg caa tgatggagat acagattgca     1991
Pro Thr Asp Pro Val Gly Gly Asn Leu Gln
                535                 540
aaaagtggtc ttggtgaggg aagagggcag ttttttggta atgaggttcc gttttcttta 2051
atgttaatag caagtgtggt taaaaagggg ttgtctagtt tataggtttt gcagatctca 2111
agtatattca tttgggtgat catgttttca gctcagttat tgcttttggt cattgctgac 2171
catcaatttc tgtggggaat tcctataggt tttctcccta acagttcttt tcttcatctt 2231
tttgcaattt atcgaaacac caaatgggtg tatattctgt aagcttgtgg catagctagc 2291
ttaattgtct tgtaaaattt cctacaggtt agagattggt tcttgatatg tagatttcat 2351
atgattgtaa cattcccatt tctcagaaaa gaaactataa tataaaattt ctggtggctg 2411
tcgcccgtgc tc                                                     2423
```

<210> 15
<211> 540
<212> PRT
<213> Petunia hybrida

<223> Amino acid sequence of protein regulating the pH of vacuoles

<400> 15

```
Met Ala Phe Asp Phe Gly Thr Leu Leu Gly Asn Val Asp Arg Leu Ser
                5                  10                  15
```

```
Thr Ser Asp His Gln Ser Val Val Ser Ile Asn Leu Phe Val Ala Leu
                20                  25                  30
Ile Cys Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp
            35                  40                  45
Met Asn Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Ile
        50                  55                  60
Val Ile Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe
 65                  70                  75                  80
Ser Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn
                85                  90                  95
Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr
            100                 105                 110
Ile Met Leu Phe Gly Ala Leu Gly Thr Leu Ile Ser Phe Ile Ile Ile
        115                 120                 125
Ser Leu Gly Ala Ile Gly Ile Phe Lys Lys Met Asn Ile Gly Ser Leu
    130                 135                 140
Glu Ile Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ser Ala Thr Asp
145                 150                 155                 160
Ser Val Cys Thr Leu Gln Val Leu Asn Gln Asp Glu Thr Pro Leu Leu
                165                 170                 175
Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val
            180                 185                 190
Val Leu Phe Asn Ala Ile Gln Asn Phe Asp Leu Ser His Ile Asp Thr
        195                 200                 205
Gly Lys Ala Met Glu Leu Val Gly Asn Phe Leu Tyr Leu Phe Ala Ser
    210                 215                 220
Ser Thr Ala Leu Gly Val Ala Ala Gly Leu Leu Ser Ala Tyr Ile Ile
225                 230                 235                 240
Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile
                245                 250                 255
Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr
            260                 265                 270
Leu Ser Ala Ile Leu Thr Val Phe Phe Ser Gly Ile Val Met Ser His
        275                 280                 285
Tyr Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His
    290                 295                 300
```

```
Thr Phe Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr
305                 310                 315                 320
Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp
                325                 330                 335
Ser Pro Gly Ile Ser Val Gln Val Ser Ser Ile Leu Leu Gly Leu Val
            340                 345                 350
Leu Val Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn
        355                 360                 365
Leu Thr Lys Lys Thr Pro Glu Ala Lys Ile Ser Phe Asn Gln Gln Val
    370                 375                 380
Thr Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu
385                 390                 395                 400
Ala Tyr Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn
                405                 410                 415
Ala Ile Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val
            420                 425                 430
Val Phe Gly Leu Met Thr Lys Pro Leu Ile Arg Ile Leu Leu Pro Ser
        435                 440                 445
His Lys His Leu Ser Arg Met Ile Ser Ser Glu Pro Thr Thr Pro Lys
    450                 455                 460
Ser Phe Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp
465                 470                 475                 480
Leu Glu Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser
                485                 490                 495
Thr Pro Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala
            500                 505                 510

Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Ala Pro
        515                 520                 525
Gly Ser Pro Thr Asp Pro Val Gly Gly Asn Leu Gln
    530                 535                 540
```

<210> 16
<211> 2553
<212> DNA

<213> Nierembergia hybrida

<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

<400> 16

```
aattattatt atttctctcc aactctcatt tctcagtttg ttgtgacttt ttcagagctt   60
gaagttcagt taattcattt tccaatatat tgattgtttt catttgagcg cgagaggatt  120
tcgtcttctc aatctgcttt caaatccttt ttgtttgtga tattcgatat tattcactca  180
gtttacctta atatttcctc gcactttctg aattcgagtg ctttgaagtg tgttggattt  240
cgaaaagcgg aagaaaattc agcaaaaacg ctgttgctga atttgcagca gtttgagttt  300
ttgctaaata gctaagatct gattgaattt ttcactggtg cttataggga aattcgacgt  360
cgttttgact gcaatatttg tccgtgattc ggactttgtt gaaattttgc tatttgaaat  420
ttgaatgtaa ggttgtcata gctttgccac tcggaaatac agtcagtgag aaagaaaaaa  480
aactgtgtag tgtttttcc acaagtattt ggtgaattga ggttcttgaa atg gcg       536
                                                        Met Ala

ttt gac ttt ggg act ctg ctg gga aag atg aac aac tta aca act tct     584
Phe Asp Phe Gly Thr Leu Leu Gly Lys Met Asn Asn Leu Thr Thr Ser
  5                  10                  15

gat cat caa tca gtg gtg tcg gta aac ttg ttt gtt gca ctt att tgc     632
Asp His Gln Ser Val Val Ser Val Asn Leu Phe Val Ala Leu Ile Cys
     20                  25                  30

gcg tgt att gtg atc ggt cat tta ttg gag gaa aac aga tgg atg aat     680
Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn
 35                  40                  45                  50

gag tcc ata act gcc ctt gtg att ggt agt tgc act gga gtc atc att     728
Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Val Ile Ile
                 55                  60                  65

cta cta ata agt gga gga aag aac tca cat att tta gtg ttc agc gaa     776
Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe Ser Glu
             70                  75                  80

gat ctt ttc ttc att tac ctt ctt cca ccg atc att ttt aat gct ggg     824
Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala Gly
         85                  90                  95
```

```
ttc cag gtg aaa aag aaa tca ttc ttc cgc aat ttc agt act atc atg    872
Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr Ile Met
    100                 105                 110
ctc ttt ggg gca gtt ggc acc ttg ata tcg ttc att att ata tca gcg    920
Leu Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Ala
115                 120                 125                 130
ggt gct att ggc att ttc aag aaa atg gat att gga cac ctt gaa att    968
Gly Ala Ile Gly Ile Phe Lys Lys Met Asp Ile Gly His Leu Glu Ile
                135                 140                 145
gga gat tac ctt gca att gga gca atc ttt gct gca aca gat tct gta   1016
Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser Val
            150                 155                 160
tgc acc tta caa gtg ctt aat cag gaa gaa aca ccg tta ttg tac agt   1064
Cys Thr Leu Gln Val Leu Asn Gln Glu Glu Thr Pro Leu Leu Tyr Ser
        165                 170                 175
cta gtg ttt gga gaa ggt gtt gtg aat gat gcc aca tct gta gtg ctg   1112
Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu
    180                 185                 190
ttc aat gct gtc cag aac ttt gac tta tct cat atc agc aca ggc aaa   1160
Phe Asn Ala Val Gln Asn Phe Asp Leu Ser His Ile Ser Thr Gly Lys
195                 200                 205                 210
gct ctg caa tta att gga aac ttt cta tac ttg ttt gcc tcg agc act   1208
Ala Leu Gln Leu Ile Gly Asn Phe Leu Tyr Leu Phe Ala Ser Ser Thr
                215                 220                 225
ttc cta ggg gtt gct gtt ggc cta cta agt gcc ttt ata att aag aaa   1256
Phe Leu Gly Val Ala Val Gly Leu Leu Ser Ala Phe Ile Ile Lys Lys
            230                 235                 240
ctc tac ttt gga agg cac tcg act gat cgt gag gtt gct ata atg ata   1304
Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile
        245                 250                 255
ctc atg gcg tac cta tca tac atg ctt gct gaa tta ttc tat tta agt   1352
Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr Leu Ser
    260                 265                 270
gga atc ctc act gtg ttt ttc tgt ggg atc gtg atg tct cac tat acc   1400
Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr Thr
275                 280                 285                 290
```

```
tgg cat aat gtg act gag agc tca aga gtc act acc aag cac acg ttt   1448
Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His Thr Phe
                295                 300                 305
gct aca tta tca ttt att gct gaa ata ttc ata ttc ctt tat gtt ggt   1496
Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly
            310                 315                 320
atg gat gct ttg gac att gag aag tgg aag ttt gta agc gac agc ccc   1544
Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp Ser Pro
        325                 330                 335
gga aca tca att aag gtc agc tca att ctg cta ggt ctt gtt ttg gtt   1592
Gly Thr Ser Ile Lys Val Ser Ser Ile Leu Leu Gly Leu Val Leu Val
    340                 345                 350
gga agg gga gcc ttt gtt ttc ccc ttg tca ttc ttg tcc aac ttg acc   1640
Gly Arg Gly Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Thr
355                 360                 365                 370
aag aaa aat cct gag gac aag att agc ttt aac cag cag gtt aca ata   1688
Lys Lys Asn Pro Glu Asp Lys Ile Ser Phe Asn Gln Gln Val Thr Ile
                375                 380                 385
tgg tgg gct ggg ctt atg cga ggt gct gtt tct atg gcc ctt gct tat   1736
Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr
            390                 395                 400
aat cag ttt acc agg gga ggt cat act cag tta cgt gcc aat gca ata   1784
Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn Ala Ile
        405                 410                 415
atg atc acg agt act atc act gtt gtc ctt ttc agc aca gtg gta ttt   1832
Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val Val Phe
    420                 425                 430
ggg ttg atg aca aaa cct tta att cta tta ttg cta ccc tca caa aaa   1880
Gly Leu Met Thr Lys Pro Leu Ile Leu Leu Leu Leu Pro Ser Gln Lys
435                 440                 445                 450
cac ttg atc aga atg atc tcc tct gaa ccg atg act cca aaa tcc ttc   1928
His Leu Ile Arg Met Ile Ser Ser Glu Pro Met Thr Pro Lys Ser Phe
                455                 460                 465
att gtg cca ctt ctt gac agc aca caa gac tca gaa gct gat ctg ggc   1976
Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp Leu Gly
            470                 475                 480
```

```
cga cat gta ccc cgt ccc cac agt ttg cgg atg ctc ctg tca acc cca   2024
Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser Thr Pro
        485                 490                 495
tct cac acg gta cat tac tac tgg aga aaa ttt gac aat gca ttc atg   2072
Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met
    500             505                 510
cgt cct gtt ttc ggt gga cga ggt ttt gta cct ttt gtt cca gga tca   2120
Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Val Pro Gly Ser
515             520                 525                 530
cct act gaa ccg gtc gaa ccg acc gaa cca aga cca gcc gaa tca aga   2168
Pro Thr Glu Pro Val Glu Pro Thr Glu Pro Arg Pro Ala Glu Ser Arg
                535                 540                 545
cca acc gaa cca act gat gag tgattacact gatggagatg caggttgcac      2219
Pro Thr Glu Pro Thr Asp Glu
            550
taaagtccca ctggccttgg agaaggacga aggcagtttt ttgggtttga ggttttgttt 2279
actgttaata gttttcgaat gtggttaaaa aagggttgtc tagtttttat atataggtcg 2339
cagatacgta atttcagctc agttcccgag gtgaacccct tagaggtttt cttcctgacg 2399
gttttttcttc ttttttgtaa tttatcaaaa acaccaaatg ggtgtatatt ctttaagctt 2459
gtagcttaat taccttataa gcatgtggta gcgttcgtgt aatatgtaaa atttccattg 2519
ccagaaaaga aacttccata caatatttct gccg                              2553
```

<210> 17
<211> 553
<212> PRT
<213> Nierembergia hybrida

<223> Amino acid sequence of protein regulating the pH of vacuoles

<400> 17

```
Met Ala Phe Asp Phe Gly Thr Leu Leu Gly Lys Met Asn Asn Leu Thr
                5                  10                  15
Thr Ser Asp His Gln Ser Val Val Ser Val Asn Leu Phe Val Ala Leu
            20                  25                  30
```

Ile Cys Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp
35 40 45
Met Asn Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Val
50 55 60
Ile Ile Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe
65 70 75 80
Ser Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn
85 90 95
Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr
100 105 110
Ile Met Leu Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile
115 120 125
Ser Ala Gly Ala Ile Gly Ile Phe Lys Lys Met Asp Ile Gly His Leu
130 135 140
Glu Ile Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp
145 150 155 160
Ser Val Cys Thr Leu Gln Val Leu Asn Gln Glu Glu Thr Pro Leu Leu
165 170 175

Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val
180 185 190
Val Leu Phe Asn Ala Val Gln Asn Phe Asp Leu Ser His Ile Ser Thr
195 200 205
Gly Lys Ala Leu Gln Leu Ile Gly Asn Phe Leu Tyr Leu Phe Ala Ser
210 215 220
Ser Thr Phe Leu Gly Val Ala Val Gly Leu Leu Ser Ala Phe Ile Ile
225 230 235 240
Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile
245 250 255
Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr
260 265 270
Leu Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His
275 280 285
Tyr Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His
290 295 300

```
Thr Phe Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr
305                 310                 315                 320
Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp
                325                 330                 335
Ser Pro Gly Thr Ser Ile Lys Val Ser Ser Ile Leu Leu Gly Leu Val
            340                 345                 350
Leu Val Gly Arg Gly Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn
        355                 360                 365
Leu Thr Lys Lys Asn Pro Glu Asp Lys Ile Ser Phe Asn Gln Gln Val
    370                 375                 380

Thr Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu
385                 390                 395                 400
Ala Tyr Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn
                405                 410                 415
Ala Ile Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val
            420                 425                 430
Val Phe Gly Leu Met Thr Lys Pro Leu Ile Leu Leu Leu Leu Pro Ser
        435                 440                 445
Gln Lys His Leu Ile Arg Met Ile Ser Ser Glu Pro Met Thr Pro Lys
    450                 455                 460
Ser Phe Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp
465                 470                 475                 480
Leu Gly Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser
                485                 490                 495
Thr Pro Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala
            500                 505                 510
Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Val Pro
        515                 520                 525
Gly Ser Pro Thr Glu Pro Val Glu Pro Thr Glu Pro Arg Pro Ala Glu
    530                 535                 540
Ser Arg Pro Thr Glu Pro Thr Asp Glu
545                 550
```

<210> 18
<211> 2361

<212> DNA
<213> Torenia hybrida

<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

<400> 18

```
gttggagatt ccgagctgca gcatcacctt gcttatgtaa gctttaaaag tatcagaatt   60
gaatatcgac cactggaaag tgttttagga cttggattct tatctattga gcttgtttga  120
aggtgaaaaa aggctcgatc tcgttcctct atagttggtt ttctggagtt gcaagcgact  180
ctactcggaa tctctttccg ccttattgga agctctgctt tactaaaaaa agtttgtctt  240
tttatctctg attcatcata aaatctgcgg gagattcaga agcggagatc tggtgcccag  300
agcaggagtt tcaactttga gcccgtttat atttataaac aaattccgag tccaaagatt  360
gaactttgaa ataatcaaat aatcaagcaa gcaat atg ggg ttt gaa tct gta      413
                                      Met Gly Phe Glu Ser Val
                                                        5
att aag cta gcg gca agt gaa act gac aat ttg tgg agc tct ggt cac     461
Ile Lys Leu Ala Ala Ser Glu Thr Asp Asn Leu Trp Ser Ser Gly His
            10                  15                  20
ggt tca gtg gtc gct ata acc tta ttt gtc act ctt ctc tgc aca tgt     509
Gly Ser Val Val Ala Ile Thr Leu Phe Val Thr Leu Leu Cys Thr Cys
        25                  30                  35
ata gtg att ggt cat ctt ctg gag gaa aac cgt tgg atg aat gaa tct     557
Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn Glu Ser
    40                  45                  50
atc att gcc ctc ata att ggt tta gcc acg gga gtt ata atc ctg tta     605
Ile Ile Ala Leu Ile Ile Gly Leu Ala Thr Gly Val Ile Ile Leu Leu
 55                  60                  65                  70
ata agt ggt gga aaa agc tcc cat ctc ttg gtg ttc agt gag gat ctt     653
Ile Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser Glu Asp Leu
                75                  80                  85

ttc ttc atc tat gcg ctg cca cca atc att ttt aat gcg ggg ttc caa     701
Phe Phe Ile Tyr Ala Leu Pro Pro Ile Ile Phe Asn Ala Gly Phe Gln
            90                  95                  100
```

```
gta aaa aag aaa tca ttc ttt cgc aat ttc gca act ata atg atg ttt    749
Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ala Thr Ile Met Met Phe
        105                 110                 115
gga gca gtt ggt acc ttg ata tcc ttc atc atc att tca ctc ggt aca    797
Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Leu Gly Thr
    120                 125                 130
att gca ttc ttc ccc aaa atg aac atg aga ctt gga gtt gga gat tat    845
Ile Ala Phe Phe Pro Lys Met Asn Met Arg Leu Gly Val Gly Asp Tyr
135                 140                 145                 150
ctt gct att gga gct att ttt gct gca aca gac tca gtt tgc aca tta    893
Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser Val Cys Thr Leu
                155                 160                 165
cag gtg cta agc cag gac gaa aca cca ctg ttg tac agt cta gtg ttt    941
Gln Val Leu Ser Gln Asp Glu Thr Pro Leu Leu Tyr Ser Leu Val Phe
            170                 175                 180
ggc gag ggt gtt gta aat gac gcg act tca gtg gtc cta ttt aat gca    989
Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu Phe Asn Ala
        185                 190                 195
gta cag aac ttc gac ctg cct cat atg tct act gct aaa gct ttc gag   1037
Val Gln Asn Phe Asp Leu Pro His Met Ser Thr Ala Lys Ala Phe Glu
    200                 205                 210
ctt gtt gga aac ttc ttt tat tta ttt gct aca agc act gtg ctg ggt   1085
Leu Val Gly Asn Phe Phe Tyr Leu Phe Ala Thr Ser Thr Val Leu Gly
215                 220                 225                 230
gtt ctg act gga ttg ctt agt gca tac atc ata aaa aag ctc tat ttt   1133
Val Leu Thr Gly Leu Leu Ser Ala Tyr Ile Ile Lys Lys Leu Tyr Phe
                235                 240                 245
gga agg cac tcc act gat cgc gag gtt gcc ata atg ata ctc atg gct   1181
Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile Leu Met Ala
            250                 255                 260
tat ctg tcg tat atg tta gct gaa tta ttc gat ttg agc ggt atc ctc   1229
Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Asp Leu Ser Gly Ile Leu
        265                 270                 275
acc gtg ttc ttc tgt gga att gtg atg tcg cac tat aca tgg cac aat   1277
Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr Thr Trp His Asn
    280                 285                 290
```

```
gtc act gaa aac tca aga gtt acc acc aag cat aca ttt gcg aca ttg   1325
Val Thr Glu Asn Ser Arg Val Thr Thr Lys His Thr Phe Ala Thr Leu
295                 300                 305                 310
tca ttt gtt gct gaa ata ttt ata ttt ctg tat gtt ggc atg gat gct   1373
Ser Phe Val Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly Met Asp Ala
                315                 320                 325
tta gac att gag aaa tgg aga ttc gta agc ggc agc atg aca aca tct   1421
Leu Asp Ile Glu Lys Trp Arg Phe Val Ser Gly Ser Met Thr Thr Ser
            330                 335                 340
gca gct gtc agt gca act ctg ctg gga ttg gtt ttg ctc tca aga gca   1469
Ala Ala Val Ser Ala Thr Leu Leu Gly Leu Val Leu Leu Ser Arg Ala
        345                 350                 355
gcc ttt gta ttc cct tta tca ttt ctc tcc aat ctg gcc aaa aag tcc   1517
Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Ala Lys Lys Ser
    360                 365                 370
cca ctc gaa aaa atc agt ctc agg cag caa att ata ata tgg tgg gct   1565
Pro Leu Glu Lys Ile Ser Leu Arg Gln Gln Ile Ile Ile Trp Trp Ala
375                 380                 385                 390
ggt ctt atg cgc gga gcc gtt tcc atg gct ctt gct tac aag cag ttt   1613
Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr Lys Gln Phe
                395                 400                 405
act aga gaa ggt ctc aca gtg gaa cgt gaa aat gcc ata ttc atc acc   1661
Thr Arg Glu Gly Leu Thr Val Glu Arg Glu Asn Ala Ile Phe Ile Thr
            410                 415                 420
agt aca atc acc att gtg ctc ttc agc act gtg gtg ttt ggt ttg atg   1709
Ser Thr Ile Thr Ile Val Leu Phe Ser Thr Val Val Phe Gly Leu Met
        425                 430                 435
acg aag ccc ctc atc aat tta ctg ata ccc tca cca aag ctt aac aga   1757
Thr Lys Pro Leu Ile Asn Leu Leu Ile Pro Ser Pro Lys Leu Asn Arg
    440                 445                 450
tcg gtc tct tca gaa ccg ctg act cca aac tcc atc aca atc cca ctt   1805
Ser Val Ser Ser Glu Pro Leu Thr Pro Asn Ser Ile Thr Ile Pro Leu
455                 460                 465                 470
ctc ggg gaa agt cag gac tct gtg gcc gaa cta ttc agc atc aga ggt   1853
Leu Gly Glu Ser Gln Asp Ser Val Ala Glu Leu Phe Ser Ile Arg Gly
                475                 480                 485
```

```
caa act tca caa ggt ggc gaa ccc gtt gct cga ccg agc agc cta cgc   1901
Gln Thr Ser Gln Gly Gly Glu Pro Val Ala Arg Pro Ser Ser Leu Arg
            490                 495                 500
atg tta ctc aca aag ccc act cat acg gtg cac tat tat tgg aga aaa   1949
Met Leu Leu Thr Lys Pro Thr His Thr Val His Tyr Tyr Trp Arg Lys
        505                 510                 515
ttc gac aat gct ttt atg cgt ccg gtc ttt ggt ggg cgt ggc ttt gta   1997
Phe Asp Asn Ala Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val
    520                 525                 530
cca tat gtt ccc ggt tca ccg act gaa cga agc gtt cgc aac tgg gaa   2045
Pro Tyr Val Pro Gly Ser Pro Thr Glu Arg Ser Val Arg Asn Trp Glu
535                 540                 545                 550
gaa gag acc aaa cag taaaaagatt ttcttgtgtg aatgatggtg aagagattag   2100
Glu Glu Thr Lys Gln
                555
attctttgga tattcgtttt tcttatttct aatgtgtcac ctgggaagtt gttgaatgaa 2160
attatattat cgtctggttt tcgactttgc gcttgtggaa ggaatatttc ttctggattt 2220
ttgcatggaaa cctcaatgat agggggtgtg atatttttgt tagaaactga gtcgtttgat 2280
gtatattgtt ggtaatgcag ctgggttttg ttttgtatgt atagtcatca agtgtgtatt 2340
tattcatatt gttatgcagt c                                          2361
```

<210> 19
<211> 555
<212> PRT
<213> Torenia hybrida

<223> Amino acid sequence of protein regulating the pH of vacuoles

<400> 19

```
Met Gly Phe Glu Ser Val Ile Lys Leu Ala Ala Ser Glu Thr Asp Asn
                5                  10                  15
Leu Trp Ser Ser Gly His Gly Ser Val Val Ala Ile Thr Leu Phe Val
            20                  25                  30
Thr Leu Leu Cys Thr Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn
        35                  40                  45
```

```
Arg Trp Met Asn Glu Ser Ile Ile Ala Leu Ile Ile Gly Leu Ala Thr
      50                  55                  60
Gly Val Ile Ile Leu Leu Ile Ser Gly Gly Lys Ser Ser His Leu Leu
 65                  70                  75                  80
Val Phe Ser Glu Asp Leu Phe Phe Ile Tyr Ala Leu Pro Pro Ile Ile
                 85                  90                  95
Phe Asn Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe
            100                 105                 110
Ala Thr Ile Met Met Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile
        115                 120                 125
Ile Ile Ser Leu Gly Thr Ile Ala Phe Phe Pro Lys Met Asn Met Arg
    130                 135                 140
Leu Gly Val Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr
145                 150                 155                 160
Asp Ser Val Cys Thr Leu Gln Val Leu Ser Gln Asp Glu Thr Pro Leu
                165                 170                 175
Leu Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser
            180                 185                 190
Val Val Leu Phe Asn Ala Val Gln Asn Phe Asp Leu Pro His Met Ser
        195                 200                 205
Thr Ala Lys Ala Phe Glu Leu Val Gly Asn Phe Phe Tyr Leu Phe Ala
    210                 215                 220
Thr Ser Thr Val Leu Gly Val Leu Thr Gly Leu Leu Ser Ala Tyr Ile
225                 230                 235                 240
Ile Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala
                245                 250                 255

Ile Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe
            260                 265                 270
Asp Leu Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser
        275                 280                 285
His Tyr Thr Trp His Asn Val Thr Glu Asn Ser Arg Val Thr Thr Lys
    290                 295                 300
His Thr Phe Ala Thr Leu Ser Phe Val Ala Glu Ile Phe Ile Phe Leu
305                 310                 315                 320
```

```
Tyr Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Arg Phe Val Ser
                325                 330                 335
Gly Ser Met Thr Thr Ser Ala Ala Val Ser Ala Thr Leu Leu Gly Leu
            340                 345                 350
Val Leu Leu Ser Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser
        355                 360                 365
Asn Leu Ala Lys Lys Ser Pro Leu Glu Lys Ile Ser Leu Arg Gln Gln
    370                 375                 380
Ile Ile Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala
385                 390                 395                 400
Leu Ala Tyr Lys Gln Phe Thr Arg Glu Gly Leu Thr Val Glu Arg Glu
                405                 410                 415
Asn Ala Ile Phe Ile Thr Ser Thr Ile Thr Ile Val Leu Phe Ser Thr
            420                 425                 430
Val Val Phe Gly Leu Met Thr Lys Pro Leu Ile Asn Leu Leu Ile Pro
        435                 440                 445
Ser Pro Lys Leu Asn Arg Ser Val Ser Ser Glu Pro Leu Thr Pro Asn
    450                 455                 460

Ser Ile Thr Ile Pro Leu Leu Gly Glu Ser Gln Asp Ser Val Ala Glu
465                 470                 475                 480
Leu Phe Ser Ile Arg Gly Gln Thr Ser Gln Gly Gly Glu Pro Val Ala
                485                 490                 495
Arg Pro Ser Ser Leu Arg Met Leu Leu Thr Lys Pro Thr His Thr Val
            500                 505                 510
His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met Arg Pro Val Phe
        515                 520                 525
Gly Gly Arg Gly Phe Val Pro Tyr Val Pro Gly Ser Pro Thr Glu Arg
    530                 535                 540
Ser Val Arg Asn Trp Glu Glu Glu Thr Lys Gln
545                 550                 555
```

<210> 20
<211> 6298
<212> DNA
<213> Ipomea nil

<223> Nucleotide sequence of promoter region of gene encoding for protein regulating the pH of vacuoles

<400> 20

gatctcagtc tgtggatgtc ctagagacat tcatatttga agttgagagt tagctaaata 60
gaaaggtaaa gacaattgga tcatataaag gtgatgagta ttatgggaaa ccatcaaaag 120
ttgggcaaat tcccgtccca tttaagaaat tcctcgaatc taaaggcatt tgtgcataat 180
acacaatgtc aggcacacca caacaaaatg gtgtggaaga aaggtgaaat cgtactctaa 240
gggaaatggt taggagttag gtaaataatt gtacattgct tgtttcattg tggatatatg 300
cattaaaaac aacagcatac ttactcaata gggttcctag taaggttgtt ttttaaaaca 360
ccttatgaac tgtggacaag aaggaaacct agtttgagac atcttcacat tcagggttgt 420
caagctggag tgatgatata taatccacat gaaaataaat tatggatatc cagaaccatt 480
aatggttatt tcattggata tccagaaagg tctaaaggta catgtttatt gtcctaatca 540
taagtacgag gattgttgag tctggtaatg ctcgcttcat ttaaaatggc gaagtcagtg 600
ggagtgtggg agctcgtaat gttaaaatta aggagtcatt gatggttcta gattcatcaa 660
gtgatccttt tttcctgttg ttgttcctat tgttgcagtg cagtgtagcc ttatggaaat 720
actttggaac aacagcaact agatgctcaa attccacatg aggaagctat tgtaaatgaa 780
gatgaggttg aaactcaaga tgatgatcaa gtgaaatctc agcaggaagt gacattaagg 840
aggtctacta tagatagaaa agatcaacca ttcttgatga ctatattgtt tatacacttg 900
agcattgata attatccagt ctcatttaac caagccatac aggataataa ttctccttga 960
ggattatttt ggttttggtg gctcattctg ttcttgagct ccaccaaatg gttgttaaaa 1020
ttacctttct gaatggtaaa ttaaaagagg aagtatacat ggattagccg taaggcttca 1080
tggccacagg aaaggaaaat ctggtatgta gattgaagat gtcgatctat ggattaaaac 1140
atgcttctag acaatggtac ttgagattgt catttggttt tgtagagatc actgttgatc 1200
ggtgtatcca cataaaggtt taatggaagc aagtttgtaa tcctagtatt acatgttaac 1260
gacattcttc ttgctgctaa taataaaagg gatgttgcgt gatgttaagg aatagctttc 1320
taagaacttt gaaatgaagg atatgggtga gacttcatat gtgattggaa taagaaatat 1380
tccgtaatag atcacatggg attttaggtt catcctagaa gactcacatt aacaaagttt 1440
tagaaagata caaactggaa atctgcaaag agggtcctgt gatacttaca aggcactaag 1500
gaccacatgc tcacctataa aatgaacgaa taacctagag gttataggtt attcggattc 1560
agactatgcc ggatgtttgg atacccgaaa atccacattc gaatatattt ttccacttgc 1620
tcgtggagca atatcttgga tgagtgtgaa ggagcctgtc attgctactt ccactataag 1680
ggcagaattt gtagcatgct ttgaggctag tagacactat aaaaattggc tgccttgtgc 1740
caacatcatt tgcatccagc tataagcatc tccattttcg aacatcattc gattcttata 1800
gctggatgaa gatgattcac tgcactttgg ttgcatattg aagctgcgat tgctatcgaa 1860

aacaaataac ctatctatat aaaacaaacg acttagattt agggaataag aaggaagata 1920
ctttttttaa aatcccaaaa ttaccttta ggtttgacct gcaaataaca ctttaagatc 1980
aaatcagata aaatgtcata atcaatgatc aaattgaata attttagtag tcgaggatca 2040
aattggtaaa atccccatag tcgagggact aaaccagtaa ttttctcgcg tttgaacgtt 2100
tgtccgaaaa ttggcattag cgatagctta attgagtttt tcaattctct aatttttaa 2160
attttgtttc ttcataaaat ccttcacttt ttcactttgc taatattttg ccgaatttat 2220
aatatttcca atttctaaag tagcagaacc ccagacgttg aactgccaat ttttttttt 2280
gtttttgttt tttgtttttt tatttcctta tccctccacc tcattttgaa gttaattatt 2340
attattaatt cattaatttt taaaatagag agactgcatt aacacaaaat tagccaatta 2400
ggtagcagaa ttaaatttaa acaaacaagt tggtttaatg taatttttgt caatttaatt 2460
tctctatttt tggacaaaaa ttaggtagac ttattaaatt aaataaacat gtttgtttaa 2520
ttttacttct tctacctaag tttgtgtcaa ttcagtctct ctattttata aattaatgaa 2580
tggttaaata taacttataa gtgcattgtg tccaaatgat cacaagagtt aggccaactt 2640
ctttttttcat ataggtgatt ctttttttcga gtattacgta cacttcagtc ttgtcaacta 2700
acacttagaa tttagttgtc attttcgaac ataggtgtca actaagtttg gtatccacta 2760
tatagcacat gtattccaag agatttaatc tcattcatca tgacaacttc tctaccaatt 2820
ctttgctcaa tcttttagtt agcgaattcg ctatattatc ctataacttt cagtatagtc 2880
aacaaaaata aaatgtattg caagaaacta tttaatagtg tatgttatgt atatgtccta 2940
tatgtctaga cttaccgtta tacatattac taatttacta tatgtccttc caattgcgaa 3000
ttgactatcg taatgcatac atattggaga tatatatttt ttctaggggt aaatgcaggt 3060
tggatcgacc cattaggcct gccccaacgc aaacttttttt tgtcgggctt ttgcggaccg 3120
gcttgcgggt tagaaaatac acagcccaag cccgtccatg cgggctcgcg ggccttattt 3180
caaaaaaaaa aaaaatacta cgtattattc tattatttta tattcaaata gtctaatata 3240
aataaataaa aaaatcgtgt ttgaaaatta ctttttttttt tatatatatt ttttaaaatt 3300
ttaatgttat atacgaagtg tgtgtaatat atatatatat atatatatat atatatatat 3360
atatatatat atatatatat attatttata ttatttatat ttatgtttat atttaaatac 3420
gggcatggct cgtcggctgg tccgttaggt ccgctctttt gtaggccatt tttttgtgtg 3480
accctaaatc gtctcaccgc gggacaagta tagggcagct tgcggacttc ggtccatttt 3540
gacatatata tatatatata tatatatata tatatatata tatatatata tatatatata 3600
tatatatata taacattaaa atttaaaaaa tatagatttt ttttaaacat gaaaaaaatt 3660
aatattattt atattaaatt atttgaatat aaaataaata atacttttttt atagcttgcg 3720
ggctggtcca taaaagcccg taaaaagaat acgttggggt tggcctaatg gaccgatcca 3780
acccgcattg acacccatag gaaaaacatc tatctccaat ttgtatgcat tacaatagtc 3840
aatttgcaat tggaagagca tatagtaatt agtaatatgt ataacggtaa gtctagacat 3900
attgaacatg tacataatac tattaaatag cttcttgcaa taggttttat ttttgttgac 3960
tatacatgaa gttataggat aatatagcga attcacaaac taaaagattg agcaatgagt 4020

tagtagaaaa gttgtcatga tggatgagat taaatctctt ggaatacatg tgctagtgga 4080
taccaaactt agctgacacc tatgttcgaa aatgacaact aaattctact acgtaagtgt 4140
tagttgacaa agtagagtgt actgaatact cgaaaaaaga atcagttatg tgaaaaaaaa 4200
aagttggcct aactcttgtg atcattttag acacaatgca cttataagtt atactagtat 4260
tttttatgcg cgatgcacaa aaaatagttg cacaatatta atacattata ttaaaatttt 4320
aaatttattt agattttaga tatttaaatt gttctaacta ataatactaa taataataat 4380
gtaaataatt tttataaatt tcagatttat atttaggtaa taattaacat ataactcaaa 4440
tatataatgt gtatatatta ttattaaggg aaaatgacac ttttttccc tgagttatat 4500
accacttttt ttcccttgag ttatttaagt ggctcttttc cccctaaaat gttaaatgga 4560
catattaacc cttaaaataa atatttcaaa taaatatttc atttattttt cttctctaac 4620
aaattattac tataatttgg ttcaaaccaa acagatacta tagcaaccaa accaaaatat 4680
tccaattaca atttagaatc aaaacgcgat atttaaagtt tattaaaatt gcaaatcgga 4740
atggtcggtt catgttccga actgaaaaaa taaaatacat ttattgttga atttagacta 4800
ttttaaaata aaaaataaaa caaaatttta aaataaagac ggttcaaaat cgcgaaccga 4860
atccggaacc gccggttcac ggttcatgat ccagtttttt tggttcataa aatttaataa 4920
attgaaatct aaatattgga ttctagatct gaatcataac cgaaaacttt taattcgatt 4980
actatagtgt ccggttcagt tcgaaccgaa ccgtggtcat tgctacatat acacaataat 5040
ttgttggaga aattaaataa ataaaatgtt tactttaagg gtagaaatgt caatttaata 5100
tttcgggggg aaaaccacca cttttaaatt aattgagggg gctaatgtgc ttatataaat 5160
ataattgagg ggaaaaagtg gtataagtat ataacttagg gggaaaaaat gtcattttcc 5220
ctattattaa tgaagaagat aagaaaatat atggtgaatg catgtgcctt tatagcataa 5280
tgtacaaaaa aaacttaacg aaaaaaacaa acataaataa ggggtataac tttcattcac 5340
acttattatg tttttagatt agatttaacc atacatgcat taatttgtaa aatagcgaga 5400
gtgaattaac acaaaattag gtagaagaag taaaattaaa caaacatgtt tatttaattt 5460
aacaagtcca cctaattttt gtccaaaaat agagaaatta aattgacaaa aattacatta 5520
aacaaacttg tttgtttaaa ttgaattctt ctacctaatt ggctaatttt gtgttaatgc 5580
agtctctcta ttttaaaaat taatgaatta ataataataa ttaacttcaa aatgaggtgg 5640
agggataagg aaataaaaaa acaaaaaaca aaaacaaaaa aaaaaaattg gcagttcaac 5700
gtctggggtt ctgctacttt aaatactgat aggagagttg tcgttcattt tacaagtatt 5760
aaggatgtac acgtattgag aatgtaggct acagaaattt tcagacagat agatacataa 5820
atccgtataa tagagacaga gaaacagaaa aagagagagt cacgttaatc ctgagatttt 5880
cctccatttg tctgaagctc ttcatccttc aacactaccc ccacatctca cctttcaagg 5940
tccaatcttt atcattcatc tttaatttcc agctctatct tgggatttgc atgtaaattt 6000
tatttatttt tcgggtttct gtttccgatc ttatgctttt gttccaaagg gtatttgatt 6060
tcatatatta tgagttttgc atgcattttc tcttttgtaa aatgaaagaa aatttgagat 6120
attggtgggt ttgatctgaa agtttgtttg tttgcagtga tttgtatgtt ttcgggaggg 6180

attggaatgg gcaacccgga tatgtgaaca gaaaccacga cattgggaaa agatttattg 6240
caaaaattgt tttgattgtt ttggattttg tggtagaaaa aggggaagaa caaaaatg 6298

SEQUENCE LISTING

<110> Iida, Shigeru
Tanaka, Sachiko
Inagaki, Yoshishige

<120> Genes Encoding Proteins Regulating the pH of Vacuoles

<130> 001560-397

<140> 09/830,123
<141> 2001-04-24

<150> PCT/JP00/05722
<151> 2000-08-24

<150> JP 11/236800
<151> 1999-08-24

<160> 20

<170> PatentIn version 3.1

<210> 1
<211> 2237
<212> DNA
<213> Ipomoea nil

<220>
<221> misc_feature
<222> (1)..(2237)
<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

<400> 1
agaatgtagg ctacagaaat tttcagacag atagatacat aaatccgtat aatagagaca 60

gagaaacaga aaaagagaga gtcacgttaa tcctgagatt ttcctccatt tgtctgaagc 120

tcttcatcct tcaacactac ccccacatct caccttttcaa gtgatttgta tgttttcggg 180

agggattgga atgggcaacc cggatatgtg aacagaaacc acgacattgg gaaaagattt 240

attgcaaaaa ttgttttgat tgttttggat tttgtggtag aaaaaggggа agaacaaaa 299

atg gcg ttc ggg ttg tct tct ttg ctc caa aat tcg gat ttg ttc acg 347
Met Ala Phe Gly Leu Ser Ser Leu Leu Gln Asn Ser Asp Leu Phe Thr
1 5 10 15

tct gat cat gct tcc gtt gtg tcg atg aac ctc ttt gtg gcg ttg ctt 395
Ser Asp His Ala Ser Val Val Ser Met Asn Leu Phe Val Ala Leu Leu
20 25 30

tgc gca tgc att gtt ctt ggc cat cta ctc gag gag aat cgc tgg gtg 443
Cys Ala Cys Ile Val Leu Gly His Leu Leu Glu Glu Asn Arg Trp Val
35 40 45

```
aac gaa tcc att act gcc ctt ata att ggt ttg tgc acc gga gtt gta    491
Asn Glu Ser Ile Thr Ala Leu Ile Ile Gly Leu Cys Thr Gly Val Val
 50                  55                  60

att ttg ctc ctt agc gga gga aag agt tca cat ctt ctc gtc ttt agc    539
Ile Leu Leu Leu Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser
 65                  70                  75                  80

gaa gat ctt ttc ttt ata tat ctc ctg cca cct ata ata ttc aat gcg    587
Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala
                 85                  90                  95

ggg ttt caa gtg aaa aag aag cag ttt ttc gtg aac ttc atg aca att    635
Gly Phe Gln Val Lys Lys Lys Gln Phe Phe Val Asn Phe Met Thr Ile
            100                 105                 110

atg ctg ttt gga gct att ggc aca ctt att agc tgt tct att ata tca    683
Met Leu Phe Gly Ala Ile Gly Thr Leu Ile Ser Cys Ser Ile Ile Ser
        115                 120                 125

ttt ggt gcg gtc aaa att ttc aag cac tta gac att gac ttt ctg gat    731
Phe Gly Ala Val Lys Ile Phe Lys His Leu Asp Ile Asp Phe Leu Asp
    130                 135                 140

ttt gga gat tat tta gca att ggt gcg ata ttt gct gca acc gat tct    779
Phe Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser
145                 150                 155                 160

gtt tgc aca ttg cag gtg ctc agt cag gat gag acg ccc cta ctt tac    827
Val Cys Thr Leu Gln Val Leu Ser Gln Asp Glu Thr Pro Leu Leu Tyr
                165                 170                 175

agt ctc gtg ttt gga gaa ggg gtc gtc aat gat gct aca tct gtg gtc    875
Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val
            180                 185                 190

ctt ttt aat gct att caa agt ttt gac atg act agt ttt gat cca aaa    923
Leu Phe Asn Ala Ile Gln Ser Phe Asp Met Thr Ser Phe Asp Pro Lys
        195                 200                 205

att ggg ctt cat ttc att gga aac ttc ttg tat tta ttt ctc tcg agc    971
Ile Gly Leu His Phe Ile Gly Asn Phe Leu Tyr Leu Phe Leu Ser Ser
    210                 215                 220

act ttt ttg ggc gtg gga att gga ctg ctt tgt gct tat att atc aaa   1019
Thr Phe Leu Gly Val Gly Ile Gly Leu Leu Cys Ala Tyr Ile Ile Lys
225                 230                 235                 240

aag cta tac ttt ggc agg cac tca acc gat cgt gag gtt gcc ctt atg   1067
Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Leu Met
                245                 250                 255

atg ctc atg tct tac ttg tct tat ata atg gcc gag tta ttc tat cta   1115
Met Leu Met Ser Tyr Leu Ser Tyr Ile Met Ala Glu Leu Phe Tyr Leu
            260                 265                 270

agc ggc ata ctt act gta ttc ttc tgt gga att gtc atg tct cat tat   1163
```

```
Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr
        275                 280                 285

acc tgg cac aat gtt acc gag agc tca agg gtc act act agg cat tcc   1211
Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Arg His Ser
    290                 295                 300

ttt gca act ctg tca ttt gtc gca gag aca ttt atc ttc ctc tat gtt   1259
Phe Ala Thr Leu Ser Phe Val Ala Glu Thr Phe Ile Phe Leu Tyr Val
305                 310                 315                 320

ggt atg gat gcc ttg gat atc gag aaa tgg aaa ttt gtg aaa aat agt   1307
Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Lys Asn Ser
                325                 330                 335

cag gga cta tca gtt gca gtg agc tca ata ttg gta ggc cta atc tta   1355
Gln Gly Leu Ser Val Ala Val Ser Ser Ile Leu Val Gly Leu Ile Leu
            340                 345                 350

gta ggc aga gct gcg ttc gta ttc ccc ttg tcg ttt tta tcc aac tta   1403
Val Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu
        355                 360                 365

gca aag aaa aac tct tcg gac aag ata tcc ttt agg caa caa ata ata   1451
Ala Lys Lys Asn Ser Ser Asp Lys Ile Ser Phe Arg Gln Gln Ile Ile
    370                 375                 380

att tgg tgg gct ggc cta atg aga ggc gcc gtc tca ata gca ctt gcg   1499
Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Ile Ala Leu Ala
385                 390                 395                 400

tat aat aag ttt aca acc tcg ggg cat acg tca ttg cac gag aac gca   1547
Tyr Asn Lys Phe Thr Thr Ser Gly His Thr Ser Leu His Glu Asn Ala
                405                 410                 415

ata atg att aca agt act gtt acg gtt gtt ctg ttc agc aca gtt gta   1595
Ile Met Ile Thr Ser Thr Val Thr Val Val Leu Phe Ser Thr Val Val
            420                 425                 430

ttc ggg ttg atg acg aag cct ctg ata aac ctt ctg cta ccc ccg cac   1643
Phe Gly Leu Met Thr Lys Pro Leu Ile Asn Leu Leu Leu Pro Pro His
        435                 440                 445

aag cag atg cca agc ggt cat tcg tca atg aca aca tcc gaa ccc agt   1691
Lys Gln Met Pro Ser Gly His Ser Ser Met Thr Thr Ser Glu Pro Ser
    450                 455                 460

agt ccg aag cac ttc acg gtg cca ctc ctg gac aac caa cct gac tca   1739
Ser Pro Lys His Phe Thr Val Pro Leu Leu Asp Asn Gln Pro Asp Ser
465                 470                 475                 480

gaa agc gat atg ata acc gga cct gag gtt gct cga cca act gcc ttg   1787
Glu Ser Asp Met Ile Thr Gly Pro Glu Val Ala Arg Pro Thr Ala Leu
                485                 490                 495

cgc atg ctg cta agg acg cca acc cac acc gtg cac cgc tac tgg cgt   1835
Arg Met Leu Leu Arg Thr Pro Thr His Thr Val His Arg Tyr Trp Arg
```

```
            500                 505                 510

aag ttt gat gat tcg ttt atg cgt ccc gtg ttt ggc ggg cgg gga ttc   1883
Lys Phe Asp Asp Ser Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe
        515                 520                 525

gtt ccg ttt gtc gcg ggc tca cca gtt gag cag agc cct aga tga        1928
Val Pro Phe Val Ala Gly Ser Pro Val Glu Gln Ser Pro Arg
    530                 535                 540

ggtacaaagt acaaacaaga cactgttgct gggtgaaata gtgtaagttg tatcatagtt 1988

gattctggtt gcccctctta tgaaatgggc tgggtgaaag tcttctcact agctaggttg 2048

cattgcattg ctacttcata aatgttttat tttattttgt aaatgttggt gcattttagg 2108

tacttgtatt aacacctcat ttgtagcata ttatttggta cagagtattt tttttatgaa 2168

acaataatgg ctgaattatc aatttggctc tatgttttga tgcttagtaa aaaaaaaaaa 2228

aaaaaaaaa                                                        2237
```

<210> 2
<211> 542
<212> PRT
<213> Ipomea nil

<220>
<221> peptide
<222> (1)..(542)
<223> Amino acid sequence of protein regulating the pH of vacuoles

<400> 2

```
Met Ala Phe Gly Leu Ser Ser Leu Leu Gln Asn Ser Asp Leu Phe Thr
  1               5                  10                  15

Ser Asp His Ala Ser Val Val Ser Met Asn Leu Phe Val Ala Leu Leu
             20                  25                  30

Cys Ala Cys Ile Val Leu Gly His Leu Leu Glu Glu Asn Arg Trp Val
         35                  40                  45

Asn Glu Ser Ile Thr Ala Leu Ile Ile Gly Leu Cys Thr Gly Val Val
     50                  55                  60

Ile Leu Leu Leu Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser
 65                  70                  75                  80

Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala
                 85                  90                  95

Gly Phe Gln Val Lys Lys Lys Gln Phe Phe Val Asn Phe Met Thr Ile
            100                 105                 110
```

Met Leu Phe Gly Ala Ile Gly Thr Leu Ile Ser Cys Ser Ile Ile Ser
115 120 125

Phe Gly Ala Val Lys Ile Phe Lys His Leu Asp Ile Asp Phe Leu Asp
130 135 140

Phe Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser
145 150 155 160

Val Cys Thr Leu Gln Val Leu Ser Gln Asp Glu Thr Pro Leu Leu Tyr
165 170 175

Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val
180 185 190

Leu Phe Asn Ala Ile Gln Ser Phe Asp Met Thr Ser Phe Asp Pro Lys
195 200 205

Ile Gly Leu His Phe Ile Gly Asn Phe Leu Tyr Leu Phe Leu Ser Ser
210 215 220

Thr Phe Leu Gly Val Gly Ile Gly Leu Leu Cys Ala Tyr Ile Ile Lys
225 230 235 240

Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Leu Met
245 250 255

Met Leu Met Ser Tyr Leu Ser Tyr Ile Met Ala Glu Leu Phe Tyr Leu
260 265 270

Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr
275 280 285

Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Arg His Ser
290 295 300

Phe Ala Thr Leu Ser Phe Val Ala Glu Thr Phe Ile Phe Leu Tyr Val
305 310 315 320

Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Lys Asn Ser
325 330 335

Gln Gly Leu Ser Val Ala Val Ser Ser Ile Leu Val Gly Leu Ile Leu
340 345 350

Val Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu
355 360 365

Ala Lys Lys Asn Ser Ser Asp Lys Ile Ser Phe Arg Gln Gln Ile Ile
370 375 380

Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Ile Ala Leu Ala
385 390 395 400

Tyr Asn Lys Phe Thr Thr Ser Gly His Thr Ser Leu His Glu Asn Ala
405 410 415

Ile Met Ile Thr Ser Thr Val Thr Val Val Leu Phe Ser Thr Val Val
420 425 430

Phe Gly Leu Met Thr Lys Pro Leu Ile Asn Leu Leu Leu Pro Pro His
435 440 445

Lys Gln Met Pro Ser Gly His Ser Ser Met Thr Thr Ser Glu Pro Ser
450 455 460

Ser Pro Lys His Phe Thr Val Pro Leu Leu Asp Asn Gln Pro Asp Ser
465 470 475 480

Glu Ser Asp Met Ile Thr Gly Pro Glu Val Ala Arg Pro Thr Ala Leu
485 490 495

Arg Met Leu Leu Arg Thr Pro Thr His Thr Val His Arg Tyr Trp Arg
500 505 510

Lys Phe Asp Asp Ser Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe
515 520 525

Val Pro Phe Val Ala Gly Ser Pro Val Glu Gln Ser Pro Arg
530 535 540

<210> 3
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> MseI adaptor

<400> 3
gacgatgagt cctgag 16

<210> 4
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> MseI adaptor

<400> 4
tactcaggac tcat 14

<210> 5
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> TIR primer

<400> 5
tgtgcatttt tcttgtagtg 20

<210> 6
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> MseI primer

<400> 6
gatgagtcct gagtaa 16

<210> 7
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> TIR+N primer

<220>
<221> misc_feature
<222> (19)..(19)
<223> Nucleotide 19 = "n" wherein "n" = any nucleotide

<400> 7
tgtgcatttt tcttgtagn 19

<210> 8
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> MseI+N primer

<220>
<221> misc_feature
<222> (17)..(17)
<223> Nucleotide 17 = "n" wherein "n" = any nucleotide

<400> 8
gatgagtcct gagtaan 17

<210> 9
<211> 130
<212> DNA
<213> Artificial Sequence

<220>

<223> MseI+N primer

<400> 9
tgagcatttt tcttgtagtg ctgagatttt cctccatttg tctgaagctc ttcatccttc 60

aacactaccc ccacatctca cctttcaagg tccaatcttt atcattcatc tttactcagg 120

actcatcgtc 130

<210> 10
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> PR-5 primer

<400> 10
gggatccaac aaaaatggct gtcggg 26

<210> 11
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> PR-3 primer

<400> 11
gggtcgacta agcatcaaaa catagagcc 29

<210> 12
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> CBSC1-linker

<400> 12
cgatagatct gggggtcgac at 22

<210> 13
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> CBSC2-linker

<400> 13
cgatgtcgac ccccagatct at 22

<210> 14
<211> 2423
<212> DNA
<213> Petunia hybrida

<220>
<221> misc_feature
<222> (1)..(2423)
<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

<400> 14
attgcgcttc gtattttact gctgaatgaa atcgtgtttt tttattcagt tcgttgttat 60

taatttcaga gttttttta ttaaaggtgt gtttggttga agaaattgta tttgctgaat 120

tttgcagaag tttttgagtt tttgctaaac tattgtgaga tctgattttg aatttttcca 180

gtggtgtttt aagctcaatt cgacgtcgtt tttactggaa ttctgatcag taaatagggc 240

tattttgatg taaggttgtg aaagtttaca gtttggaagt tgagttagtg aaaaagggga 300

aactttattg tgatattttc acaagtattt ggtgaattca ggttattgag a atg gct 357
Met Ala

ttt gat ttt ggg acg ttg ttg gga aat gta gac agg tta tcg aca tct 405
Phe Asp Phe Gly Thr Leu Leu Gly Asn Val Asp Arg Leu Ser Thr Ser
5 10 15

gat cat caa tca gtt gtg tcg ata aac tta ttc gtt gct ctt att tgc 453
Asp His Gln Ser Val Val Ser Ile Asn Leu Phe Val Ala Leu Ile Cys
20 25 30

gcg tgt att gtg atc ggt cat ttg ttg gaa gaa aac aga tgg atg aat 501
Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn
35 40 45 50

gag tcc ata act gcc tta gtg att ggt tct tgt act gga atc gtt att 549
Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Ile Val Ile
55 60 65

cta ctg ata agt gga gga aag aac tct cat att tta gtg ttc agt gaa 597
Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe Ser Glu
70 75 80

gat ctt ttc ttc att tac ctt ctt ccg cca atc att ttt aat gct ggg 645
Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala Gly
85 90 95

ttc cag gtg aaa aag aaa tcg ttc ttc cgc aat ttc agc act atc atg 693
Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr Ile Met
100 105 110

ctc ttt ggg gca ctt ggc acc ttg ata tca ttc att att ata tca tta 741
Leu Phe Gly Ala Leu Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Leu
115 120 125 130

```
ggt gcc att ggc att ttc aag aaa atg aat att gga agc ctt gaa att    789
Gly Ala Ile Gly Ile Phe Lys Lys Met Asn Ile Gly Ser Leu Glu Ile
                135                 140                 145

gga gat tac ctt gca att ggg gca atc ttc tct gct aca gat tct gta    837
Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ser Ala Thr Asp Ser Val
            150                 155                 160

tgc acc tta caa gtg ctt aat cag gat gaa aca ccc tta ttg tac agt    885
Cys Thr Leu Gln Val Leu Asn Gln Asp Glu Thr Pro Leu Leu Tyr Ser
        165                 170                 175

cta gtt ttt ggg gaa ggt gtt gtg aat gat gcc aca tct gta gtt ctg    933
Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu
    180                 185                 190

ttc aat gct atc cag aac ttt gac tta tct cac atc gac acg ggc aaa    981
Phe Asn Ala Ile Gln Asn Phe Asp Leu Ser His Ile Asp Thr Gly Lys
195                 200                 205                 210

gct atg gaa tta gtt gga aac ttt cta tac ttg ttt gcc tca agc act   1029
Ala Met Glu Leu Val Gly Asn Phe Leu Tyr Leu Phe Ala Ser Ser Thr
                215                 220                 225

gcc cta gga gtt gct gct ggc cta ctg agc gcc tat att att aaa aaa   1077
Ala Leu Gly Val Ala Ala Gly Leu Leu Ser Ala Tyr Ile Ile Lys Lys
            230                 235                 240

ctc tac ttt gga agg cac tca act gac cgt gag gtt gct ata atg ata   1125
Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile
        245                 250                 255

ctc atg gct tac cta tct tac atg ctt gct gaa tta ttc tat tta agt   1173
Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr Leu Ser
    260                 265                 270

gca atc ctc act gtg ttt ttc tct ggg atc gtg atg tct cac tac acc   1221
Ala Ile Leu Thr Val Phe Phe Ser Gly Ile Val Met Ser His Tyr Thr
275                 280                 285                 290

tgg cat aat gtg act gag agc tcg aga gtc act acc aag cac act ttt   1269
Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His Thr Phe
                295                 300                 305

gct aca tta tca ttt att gct gaa ata ttc ata ttc ctt tat gtt ggt   1317
Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly
            310                 315                 320

atg gat gct ttg gac att gag aag tgg aag ttt gta agc gac agc cct   1365
Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp Ser Pro
        325                 330                 335

gga ata tca gtt cag gtt agc tca ata ttg ctg ggt ctt gtt ttg gtt   1413
Gly Ile Ser Val Gln Val Ser Ser Ile Leu Leu Gly Leu Val Leu Val
    340                 345                 350

gga aga gca gca ttt gtt ttc cca ttg tca ttc ttg tcc aac ttg acc   1461
```

```
Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Thr
355                 360                 365                 370

aag aaa act cca gag gcg aaa att agt ttt aac cag cag gtt aca ata   1509
Lys Lys Thr Pro Glu Ala Lys Ile Ser Phe Asn Gln Gln Val Thr Ile
                375                 380                 385

tgg tgg gct gga ctt atg aga ggt gcc gtt tct atg gcc ctt gct tat   1557
Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr
            390                 395                 400

aat cag ttt acc agg gga ggt cat act cag tta cgc gca aat gca ata   1605
Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn Ala Ile
        405                 410                 415

atg atc aca agt act atc act gtt gtc ctt ttc agc aca gtc gtg ttt   1653
Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val Val Phe
    420                 425                 430

ggg ttg atg aca aaa cct ttg att aga ata ttg cta ccc tca cac aaa   1701
Gly Leu Met Thr Lys Pro Leu Ile Arg Ile Leu Leu Pro Ser His Lys
435                 440                 445                 450

cac ttg agc aga atg atc tct tct gaa cca acg acc cca aaa tcc ttc   1749
His Leu Ser Arg Met Ile Ser Ser Glu Pro Thr Thr Pro Lys Ser Phe
                455                 460                 465

att gtg cca ctt ctt gac agc aca caa gac tca gaa gct gat ctg gaa   1797
Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp Leu Glu
            470                 475                 480

cgc cat gta ccc cgt ccc cac agt ttg cgg atg ctc ctt tca acc cca   1845
Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser Thr Pro
        485                 490                 495

tct cat aca gtg cat tat tac tgg aga aag ttt gac aat gca ttc atg   1893
Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met
    500                 505                 510

cgt cca gtt ttc ggt gga cga ggt ttt gta cct ttt gct cca gga tca   1941
Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Ala Pro Gly Ser
515                 520                 525                 530

ccg aca gac cca gtt ggt gga aat ttg caa tgatggagat acagattgca     1991
Pro Thr Asp Pro Val Gly Gly Asn Leu Gln
                535                 540

aaaagtggtc ttggtgaggg aagagggcag ttttttggta atgaggttcc gttttcttta 2051

atgttaatag caagtgtggt taaaaagggg ttgtctagtt tataggtttt gcagatctca 2111

agtatattca tttgggtgat catgttttca gctcagttat tgcttttggt cattgctgac 2171

catcaatttc tgtggggaat tcctataggt tttctcccta acagttcttt tcttcatctt 2231

tttgcaattt atcgaaacac caaatgggtg tatattctgt aagcttgtgg catagctagc 2291
```

ttaattgtct tgtaaaattt cctacaggtt agagattggt tcttgatatg tagatttcat 2351

atgattgtaa cattcccatt tctcagaaaa gaaactataa tataaaattt ctggtggctg 2411

tcgcccgtgc tc 2423

<210> 15
<211> 540
<212> PRT
<213> Petunia hybrida

<220>
<221> peptide
<222> (1)..(540)
<223> Amino acid sequence of protein regulating the pH of vacuoles

<400> 15

Met Ala Phe Asp Phe Gly Thr Leu Leu Gly Asn Val Asp Arg Leu Ser
1 5 10 15

Thr Ser Asp His Gln Ser Val Val Ser Ile Asn Leu Phe Val Ala Leu
20 25 30

Ile Cys Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp
35 40 45

Met Asn Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Ile
50 55 60

Val Ile Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe
65 70 75 80

Ser Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn
85 90 95

Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr
100 105 110

Ile Met Leu Phe Gly Ala Leu Gly Thr Leu Ile Ser Phe Ile Ile Ile
115 120 125

Ser Leu Gly Ala Ile Gly Ile Phe Lys Lys Met Asn Ile Gly Ser Leu
130 135 140

Glu Ile Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ser Ala Thr Asp
145 150 155 160

Ser Val Cys Thr Leu Gln Val Leu Asn Gln Asp Glu Thr Pro Leu Leu
165 170 175

Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val
180 185 190

Val Leu Phe Asn Ala Ile Gln Asn Phe Asp Leu Ser His Ile Asp Thr
195 200 205

Gly Lys Ala Met Glu Leu Val Gly Asn Phe Leu Tyr Leu Phe Ala Ser
210 215 220

Ser Thr Ala Leu Gly Val Ala Ala Gly Leu Leu Ser Ala Tyr Ile Ile
225 230 235 240

Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile
245 250 255

Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr
260 265 270

Leu Ser Ala Ile Leu Thr Val Phe Phe Ser Gly Ile Val Met Ser His
275 280 285

Tyr Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His
290 295 300

Thr Phe Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr
305 310 315 320

Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp
325 330 335

Ser Pro Gly Ile Ser Val Gln Val Ser Ser Ile Leu Leu Gly Leu Val
340 345 350

Leu Val Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn
355 360 365

Leu Thr Lys Lys Thr Pro Glu Ala Lys Ile Ser Phe Asn Gln Gln Val
370 375 380

Thr Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu
385 390 395 400

Ala Tyr Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn
405 410 415

Ala Ile Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val
420 425 430

Val Phe Gly Leu Met Thr Lys Pro Leu Ile Arg Ile Leu Leu Pro Ser
435 440 445

His Lys His Leu Ser Arg Met Ile Ser Ser Glu Pro Thr Thr Pro Lys
450 455 460

Ser Phe Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp
465 470 475 480

Leu Glu Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser
485 490 495

```
Thr Pro Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala
            500                 505                 510

Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Ala Pro
        515                 520                 525

Gly Ser Pro Thr Asp Pro Val Gly Gly Asn Leu Gln
    530                 535                 540
```

<210> 16
<211> 2553
<212> DNA
<213> Nierembergia hybrida

<220>
<221> misc_feature
<222> (1)..(2553)
<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

<400> 16
aattattatt atttctctcc aactctcatt tctcagtttg ttgtgacttt ttcagagctt 60

gaagttcagt taattcattt tccaatatat tgattgtttt catttgagcg cgagaggatt 120

tcgtcttctc aatctgcttt caaatccttt ttgtttgtga tattcgatat tattcactca 180

gtttacctta atatttcctc gcactttctg aattcgagtg ctttgaagtg tgttggattt 240

cgaaaagcgg aagaaaattc agcaaaaacg ctgttgctga atttgcagca gtttgagttt 300

ttgctaaata gctaagatct gattgaattt ttcactggtg cttataggga aattcgacgt 360

cgttttgact gcaatatttg tccgtgattc ggactttgtt gaaattttgc tatttgaaat 420

ttgaatgtaa ggttgtcata gctttgccac tcggaaatac agtcagtgag aaagaaaaaa 480

aactgtgtag tgtttttttcc acaagtattt ggtgaattga ggttcttgaa atg gcg 536
                                                        Met Ala

ttt gac ttt ggg act ctg ctg gga aag atg aac aac tta aca act tct 584
Phe Asp Phe Gly Thr Leu Leu Gly Lys Met Asn Asn Leu Thr Thr Ser
          5   10   15

gat cat caa tca gtg gtg tcg gta aac ttg ttt gtt gca ctt att tgc 632
Asp His Gln Ser Val Val Ser Val Asn Leu Phe Val Ala Leu Ile Cys
     20   25   30

gcg tgt att gtg atc ggt cat tta ttg gag gaa aac aga tgg atg aat 680
Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn
 35   40   45   50

gag tcc ata act gcc ctt gtg att ggt agt tgc act gga gtc atc att 728
Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Val Ile Ile
                 55   60   65

```
cta cta ata agt gga gga aag aac tca cat att tta gtg ttc agc gaa    776
Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe Ser Glu
                70                  75                  80

gat ctt ttc ttc att tac ctt ctt cca ccg atc att ttt aat gct ggg    824
Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala Gly
            85                  90                  95

ttc cag gtg aaa aag aaa tca ttc ttc cgc aat ttc agt act atc atg    872
Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr Ile Met
        100                 105                 110

ctc ttt ggg gca gtt ggc acc ttg ata tcg ttc att att ata tca gcg    920
Leu Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Ala
    115                 120                 125                 130

ggt gct att ggc att ttc aag aaa atg gat att gga cac ctt gaa att    968
Gly Ala Ile Gly Ile Phe Lys Lys Met Asp Ile Gly His Leu Glu Ile
                135                 140                 145

gga gat tac ctt gca att gga gca atc ttt gct gca aca gat tct gta   1016
Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser Val
            150                 155                 160

tgc acc tta caa gtg ctt aat cag gaa gaa aca ccg tta ttg tac agt   1064
Cys Thr Leu Gln Val Leu Asn Gln Glu Glu Thr Pro Leu Leu Tyr Ser
        165                 170                 175

cta gtg ttt gga gaa ggt gtt gtg aat gat gcc aca tct gta gtg ctg   1112
Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu
    180                 185                 190

ttc aat gct gtc cag aac ttt gac tta tct cat atc agc aca ggc aaa   1160
Phe Asn Ala Val Gln Asn Phe Asp Leu Ser His Ile Ser Thr Gly Lys
195                 200                 205                 210

gct ctg caa tta att gga aac ttt cta tac ttg ttt gcc tcg agc act   1208
Ala Leu Gln Leu Ile Gly Asn Phe Leu Tyr Leu Phe Ala Ser Ser Thr
                215                 220                 225

ttc cta ggg gtt gct gtt ggc cta cta agt gcc ttt ata att aag aaa   1256
Phe Leu Gly Val Ala Val Gly Leu Leu Ser Ala Phe Ile Ile Lys Lys
            230                 235                 240

ctc tac ttt gga agg cac tcg act gat cgt gag gtt gct ata atg ata   1304
Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile
        245                 250                 255

ctc atg gcg tac cta tca tac atg ctt gct gaa tta ttc tat tta agt   1352
Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr Leu Ser
    260                 265                 270

gga atc ctc act gtg ttt ttc tgt ggg atc gtg atg tct cac tat acc   1400
Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr Thr
275                 280                 285                 290

tgg cat aat gtg act gag agc tca aga gtc act acc aag cac acg ttt   1448
```

```
Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His Thr Phe
                295                 300                 305

gct aca tta tca ttt att gct gaa ata ttc ata ttc ctt tat gtt ggt   1496
Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly
            310                 315                 320

atg gat gct ttg gac att gag aag tgg aag ttt gta agc gac agc ccc   1544
Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp Ser Pro
        325                 330                 335

gga aca tca att aag gtc agc tca att ctg cta ggt ctt gtt ttg gtt   1592
Gly Thr Ser Ile Lys Val Ser Ser Ile Leu Leu Gly Leu Val Leu Val
    340                 345                 350

gga agg gga gcc ttt gtt ttc ccc ttg tca ttc ttg tcc aac ttg acc   1640
Gly Arg Gly Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Thr
355                 360                 365                 370

aag aaa aat cct gag gac aag att agc ttt aac cag cag gtt aca ata   1688
Lys Lys Asn Pro Glu Asp Lys Ile Ser Phe Asn Gln Gln Val Thr Ile
                375                 380                 385

tgg tgg gct ggg ctt atg cga ggt gct gtt tct atg gcc ctt gct tat   1736
Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr
            390                 395                 400

aat cag ttt acc agg gga ggt cat act cag tta cgt gcc aat gca ata   1784
Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn Ala Ile
        405                 410                 415

atg atc acg agt act atc act gtt gtc ctt ttc agc aca gtg gta ttt   1832
Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val Val Phe
    420                 425                 430

ggg ttg atg aca aaa cct tta att cta tta ttg cta ccc tca caa aaa   1880
Gly Leu Met Thr Lys Pro Leu Ile Leu Leu Leu Leu Pro Ser Gln Lys
435                 440                 445                 450

cac ttg atc aga atg atc tcc tct gaa ccg atg act cca aaa tcc ttc   1928
His Leu Ile Arg Met Ile Ser Ser Glu Pro Met Thr Pro Lys Ser Phe
                455                 460                 465

att gtg cca ctt ctt gac agc aca caa gac tca gaa gct gat ctg ggc   1976
Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp Leu Gly
            470                 475                 480

cga cat gta ccc cgt ccc cac agt ttg cgg atg ctc ctg tca acc cca   2024
Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser Thr Pro
        485                 490                 495

tct cac acg gta cat tac tac tgg aga aaa ttt gac aat gca ttc atg   2072
Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met
    500                 505                 510

cgt cct gtt ttc ggt gga cga ggt ttt gta cct ttt gtt cca gga tca   2120
Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Val Pro Gly Ser
```

```
515             520                 525                 530

cct act gaa ccg gtc gaa ccg acc gaa cca aga cca gcc gaa tca aga   2168
Pro Thr Glu Pro Val Glu Pro Thr Glu Pro Arg Pro Ala Glu Ser Arg
                535                 540                 545

cca acc gaa cca act gat gag tgattacact gatggagatg caggttgcac      2219
Pro Thr Glu Pro Thr Asp Glu
            550

taaagtccca ctggccttgg agaaggacga aggcagtttt ttgggtttga ggttttgttt 2279

actgttaata gttttcgaat gtggttaaaa aagggttgtc tagtttttat atataggtcg 2339

cagatacgta atttcagctc agttcccgag gtgaacccct tagaggtttt cttcctgacg 2399

gtttttcttc ttttttgtaa tttatcaaaa acaccaaatg ggtgtatatt ctttaagctt 2459

gtagcttaat taccttataa gcatgtggta gcgttcgtgt aatatgtaaa atttccattg 2519

ccagaaaaga aacttccata caatatttct gccg                              2553


<210>  17
<211>  553
<212>  PRT
<213>  Nierembergia hybrida

<220>
<221>  peptide
<222>  (1)..(553)
<223>  Amino acid sequence of protein regulating the pH of vacuoles

<400> 17

Met Ala Phe Asp Phe Gly Thr Leu Leu Gly Lys Met Asn Asn Leu Thr
                  5                  10                  15

Thr Ser Asp His Gln Ser Val Val Ser Val Asn Leu Phe Val Ala Leu
             20                  25                  30

Ile Cys Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp
         35                  40                  45

Met Asn Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Val
     50                  55                  60

Ile Ile Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe
65                  70                  75                  80

Ser Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn
                 85                  90                  95

Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr
            100                 105                 110

Ile Met Leu Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile
```

```
        115                 120                 125

Ser Ala Gly Ala Ile Gly Ile Phe Lys Lys Met Asp Ile Gly His Leu
    130                 135                 140

Glu Ile Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp
145                 150                 155                 160

Ser Val Cys Thr Leu Gln Val Leu Asn Gln Glu Glu Thr Pro Leu Leu
                165                 170                 175


Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val
            180                 185                 190

Val Leu Phe Asn Ala Val Gln Asn Phe Asp Leu Ser His Ile Ser Thr
        195                 200                 205

Gly Lys Ala Leu Gln Leu Ile Gly Asn Phe Leu Tyr Leu Phe Ala Ser
    210                 215                 220

Ser Thr Phe Leu Gly Val Ala Val Gly Leu Leu Ser Ala Phe Ile Ile
225                 230                 235                 240

Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile
                245                 250                 255

Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr
            260                 265                 270

Leu Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His
        275                 280                 285

Tyr Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His
    290                 295                 300

Thr Phe Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr
305                 310                 315                 320

Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp
                325                 330                 335

Ser Pro Gly Thr Ser Ile Lys Val Ser Ser Ile Leu Leu Gly Leu Val
            340                 345                 350

Leu Val Gly Arg Gly Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn
        355                 360                 365

Leu Thr Lys Lys Asn Pro Glu Asp Lys Ile Ser Phe Asn Gln Gln Val
    370                 375                 380

Thr Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu
385                 390                 395                 400

Ala Tyr Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn
                405                 410                 415
```

```
Ala Ile Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val
            420                 425                 430

Val Phe Gly Leu Met Thr Lys Pro Leu Ile Leu Leu Leu Leu Pro Ser
        435                 440                 445

Gln Lys His Leu Ile Arg Met Ile Ser Ser Glu Pro Met Thr Pro Lys
    450                 455                 460

Ser Phe Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp
465             470                 475                 480

Leu Gly Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser
                485                 490                 495

Thr Pro Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala
            500                 505                 510

Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Val Pro
        515                 520                 525

Gly Ser Pro Thr Glu Pro Val Glu Pro Thr Glu Pro Arg Pro Ala Glu
    530                 535                 540

Ser Arg Pro Thr Glu Pro Thr Asp Glu
545                 550
```

<210> 18
<211> 2361
<212> DNA
<213> Torenia hybrida

<220>
<221> misc_feature
<222> (1)..(2361)
<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

<400> 18

```
gttggagatt ccgagctgca gcatcacctt gcttatgtaa gctttaaaag tatcagaatt   60

gaatatcgac cactggaaag tgttttagga cttggattct tatctattga gcttgtttga  120

aggtgaaaaa aggctcgatc tcgttcctct atagttggtt ttctggagtt gcaagcgact  180

ctactcggaa tctctttccg ccttattgga agctctgctt tactaaaaaa agtttgtctt  240

tttatctctg attcatcata aaatctgcgg gagattcaga agcggagatc tggtgcccag  300

agcaggagtt tcaactttga gcccgtttat atttataaac aaattccgag tccaaagatt  360

gaactttgaa ataatcaaat aatcaagcaa gcaat atg ggg ttt gaa tct gta      413
                                        Met Gly Phe Glu Ser Val
                                          5

att aag cta gcg gca agt gaa act gac aat ttg tgg agc tct ggt cac    461
```

```
Ile Lys Leu Ala Ala Ser Glu Thr Asp Asn Leu Trp Ser Ser Gly His
                10                  15                  20

ggt tca gtg gtc gct ata acc tta ttt gtc act ctt ctc tgc aca tgt    509
Gly Ser Val Val Ala Ile Thr Leu Phe Val Thr Leu Leu Cys Thr Cys
        25                  30                  35

ata gtg att ggt cat ctt ctg gag gaa aac cgt tgg atg aat gaa tct    557
Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn Glu Ser
    40                  45                  50

atc att gcc ctc ata att ggt tta gcc acg gga gtt ata atc ctg tta    605
Ile Ile Ala Leu Ile Ile Gly Leu Ala Thr Gly Val Ile Ile Leu Leu
55                  60                  65                  70

ata agt ggt gga aaa agc tcc cat ctc ttg gtg ttc agt gag gat ctt    653
Ile Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser Glu Asp Leu
                75                  80                  85


ttc ttc atc tat gcg ctg cca cca atc att ttt aat gcg ggg ttc caa    701
Phe Phe Ile Tyr Ala Leu Pro Pro Ile Ile Phe Asn Ala Gly Phe Gln
            90                  95                  100

gta aaa aag aaa tca ttc ttt cgc aat ttc gca act ata atg atg ttt    749
Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ala Thr Ile Met Met Phe
        105                 110                 115

gga gca gtt ggt acc ttg ata tcc ttc atc atc att tca ctc ggt aca    797
Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Leu Gly Thr
    120                 125                 130

att gca ttc ttc ccc aaa atg aac atg aga ctt gga gtt gga gat tat    845
Ile Ala Phe Phe Pro Lys Met Asn Met Arg Leu Gly Val Gly Asp Tyr
135                 140                 145                 150

ctt gct att gga gct att ttt gct gca aca gac tca gtt tgc aca tta    893
Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser Val Cys Thr Leu
                155                 160                 165

cag gtg cta agc cag gac gaa aca cca ctg ttg tac agt cta gtg ttt    941
Gln Val Leu Ser Gln Asp Glu Thr Pro Leu Leu Tyr Ser Leu Val Phe
            170                 175                 180

ggc gag ggt gtt gta aat gac gcg act tca gtg gtc cta ttt aat gca    989
Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu Phe Asn Ala
        185                 190                 195

gta cag aac ttc gac ctg cct cat atg tct act gct aaa gct ttc gag   1037
Val Gln Asn Phe Asp Leu Pro His Met Ser Thr Ala Lys Ala Phe Glu
    200                 205                 210

ctt gtt gga aac ttc ttt tat tta ttt gct aca agc act gtg ctg ggt   1085
Leu Val Gly Asn Phe Phe Tyr Leu Phe Ala Thr Ser Thr Val Leu Gly
215                 220                 225                 230

gtt ctg act gga ttg ctt agt gca tac atc ata aaa aag ctc tat ttt   1133
```

```
Val Leu Thr Gly Leu Leu Ser Ala Tyr Ile Ile Lys Lys Leu Tyr Phe
                235                 240                 245

gga agg cac tcc act gat cgc gag gtt gcc ata atg ata ctc atg gct   1181
Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile Leu Met Ala
            250                 255                 260

tat ctg tcg tat atg tta gct gaa tta ttc gat ttg agc ggt atc ctc   1229
Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Asp Leu Ser Gly Ile Leu
        265                 270                 275

acc gtg ttc ttc tgt gga att gtg atg tcg cac tat aca tgg cac aat   1277
Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr Thr Trp His Asn
    280                 285                 290

gtc act gaa aac tca aga gtt acc acc aag cat aca ttt gcg aca ttg   1325
Val Thr Glu Asn Ser Arg Val Thr Thr Lys His Thr Phe Ala Thr Leu
295                 300                 305                 310

tca ttt gtt gct gaa ata ttt ata ttt ctg tat gtt ggc atg gat gct   1373
Ser Phe Val Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly Met Asp Ala
                315                 320                 325

tta gac att gag aaa tgg aga ttc gta agc ggc agc atg aca aca tct   1421
Leu Asp Ile Glu Lys Trp Arg Phe Val Ser Gly Ser Met Thr Thr Ser
            330                 335                 340

gca gct gtc agt gca act ctg ctg gga ttg gtt ttg ctc tca aga gca   1469
Ala Ala Val Ser Ala Thr Leu Leu Gly Leu Val Leu Leu Ser Arg Ala
        345                 350                 355

gcc ttt gta ttc cct tta tca ttt ctc tcc aat ctg gcc aaa aag tcc   1517
Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Ala Lys Lys Ser
    360                 365                 370

cca ctc gaa aaa atc agt ctc agg cag caa att ata ata tgg tgg gct   1565
Pro Leu Glu Lys Ile Ser Leu Arg Gln Gln Ile Ile Ile Trp Trp Ala
375                 380                 385                 390

ggt ctt atg cgc gga gcc gtt tcc atg gct ctt gct tac aag cag ttt   1613
Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr Lys Gln Phe
                395                 400                 405

act aga gaa ggt ctc aca gtg gaa cgt gaa aat gcc ata ttc atc acc   1661
Thr Arg Glu Gly Leu Thr Val Glu Arg Glu Asn Ala Ile Phe Ile Thr
            410                 415                 420

agt aca atc acc att gtg ctc ttc agc act gtg gtg ttt ggt ttg atg   1709
Ser Thr Ile Thr Ile Val Leu Phe Ser Thr Val Val Phe Gly Leu Met
        425                 430                 435

acg aag ccc ctc atc aat tta ctg ata ccc tca cca aag ctt aac aga   1757
Thr Lys Pro Leu Ile Asn Leu Leu Ile Pro Ser Pro Lys Leu Asn Arg
    440                 445                 450

tcg gtc tct tca gaa ccg ctg act cca aac tcc atc aca atc cca ctt   1805
Ser Val Ser Ser Glu Pro Leu Thr Pro Asn Ser Ile Thr Ile Pro Leu
```

```
455                 460                 465                 470

ctc ggg gaa agt cag gac tct gtg gcc gaa cta ttc agc atc aga ggt   1853
Leu Gly Glu Ser Gln Asp Ser Val Ala Glu Leu Phe Ser Ile Arg Gly
                475                 480                 485

caa act tca caa ggt ggc gaa ccc gtt gct cga ccg agc agc cta cgc   1901
Gln Thr Ser Gln Gly Gly Glu Pro Val Ala Arg Pro Ser Ser Leu Arg
            490                 495                 500

atg tta ctc aca aag ccc act cat acg gtg cac tat tat tgg aga aaa   1949
Met Leu Leu Thr Lys Pro Thr His Thr Val His Tyr Tyr Trp Arg Lys
        505                 510                 515

ttc gac aat gct ttt atg cgt ccg gtc ttt ggt ggg cgt ggc ttt gta   1997
Phe Asp Asn Ala Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val
    520                 525                 530

cca tat gtt ccc ggt tca ccg act gaa cga agc gtt cgc aac tgg gaa   2045
Pro Tyr Val Pro Gly Ser Pro Thr Glu Arg Ser Val Arg Asn Trp Glu
535                 540                 545                 550

gaa gag acc aaa cag taaaaagatt ttcttgtgtg aatgatggtg aagagattag   2100
Glu Glu Thr Lys Gln
                555

attctttgga tattcgtttt tcttatttct aatgtgtcac ctgggaagtt gttgaatgaa 2160

attatattat cgtctggttt tcgactttgc gcttgtggaa ggaatatttc ttctggattt 2220

tgcatggaaa cctcaatgat agggggtgtg atatttttgt tagaaactga gtcgtttgat 2280

gtatattgtt ggtaatgcag ctgggttttg ttttgtatgt atagtcatca agtgtgtatt 2340

tattcatatt gttatgcagt c                                          2361


<210>  19
<211>  555
<212>  PRT
<213>  Torenia hybrida

<220>
<221>  peptide
<222>  (1)..(555)
<223>  Amino acid sequence of protein regulating the pH of vacuoles

<400>  19

Met Gly Phe Glu Ser Val Ile Lys Leu Ala Ala Ser Glu Thr Asp Asn
                5                  10                  15

Leu Trp Ser Ser Gly His Gly Ser Val Val Ala Ile Thr Leu Phe Val
            20                  25                  30

Thr Leu Leu Cys Thr Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn
        35                  40                  45
```

```
Arg Trp Met Asn Glu Ser Ile Ile Ala Leu Ile Ile Gly Leu Ala Thr
     50                  55                  60

Gly Val Ile Ile Leu Leu Ile Ser Gly Gly Lys Ser Ser His Leu Leu
 65                  70                  75                  80

Val Phe Ser Glu Asp Leu Phe Phe Ile Tyr Ala Leu Pro Pro Ile Ile
                 85                  90                  95

Phe Asn Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe
            100                 105                 110

Ala Thr Ile Met Met Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile
        115                 120                 125

Ile Ile Ser Leu Gly Thr Ile Ala Phe Phe Pro Lys Met Asn Met Arg
    130                 135                 140

Leu Gly Val Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr
145                 150                 155                 160

Asp Ser Val Cys Thr Leu Gln Val Leu Ser Gln Asp Glu Thr Pro Leu
                165                 170                 175

Leu Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser
            180                 185                 190

Val Val Leu Phe Asn Ala Val Gln Asn Phe Asp Leu Pro His Met Ser
        195                 200                 205

Thr Ala Lys Ala Phe Glu Leu Val Gly Asn Phe Phe Tyr Leu Phe Ala
    210                 215                 220

Thr Ser Thr Val Leu Gly Val Leu Thr Gly Leu Leu Ser Ala Tyr Ile
225                 230                 235                 240

Ile Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala
                245                 250                 255

Ile Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe
            260                 265                 270

Asp Leu Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser
        275                 280                 285

His Tyr Thr Trp His Asn Val Thr Glu Asn Ser Arg Val Thr Thr Lys
    290                 295                 300

His Thr Phe Ala Thr Leu Ser Phe Val Ala Glu Ile Phe Ile Phe Leu
305                 310                 315                 320

Tyr Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Arg Phe Val Ser
                325                 330                 335

Gly Ser Met Thr Thr Ser Ala Ala Val Ser Ala Thr Leu Leu Gly Leu
            340                 345                 350
```

```
Val Leu Leu Ser Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser
        355                 360                 365

Asn Leu Ala Lys Lys Ser Pro Leu Glu Lys Ile Ser Leu Arg Gln Gln
    370                 375                 380

Ile Ile Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala
385                 390                 395                 400

Leu Ala Tyr Lys Gln Phe Thr Arg Glu Gly Leu Thr Val Glu Arg Glu
                405                 410                 415

Asn Ala Ile Phe Ile Thr Ser Thr Ile Thr Ile Val Leu Phe Ser Thr
            420                 425                 430

Val Val Phe Gly Leu Met Thr Lys Pro Leu Ile Asn Leu Leu Ile Pro
        435                 440                 445

Ser Pro Lys Leu Asn Arg Ser Val Ser Ser Glu Pro Leu Thr Pro Asn
    450                 455                 460

Ser Ile Thr Ile Pro Leu Leu Gly Glu Ser Gln Asp Ser Val Ala Glu
465                 470                 475                 480

Leu Phe Ser Ile Arg Gly Gln Thr Ser Gln Gly Gly Glu Pro Val Ala
                485                 490                 495

Arg Pro Ser Ser Leu Arg Met Leu Leu Thr Lys Pro Thr His Thr Val
            500                 505                 510

His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met Arg Pro Val Phe
        515                 520                 525

Gly Gly Arg Gly Phe Val Pro Tyr Val Pro Gly Ser Pro Thr Glu Arg
    530                 535                 540

Ser Val Arg Asn Trp Glu Glu Glu Thr Lys Gln
545                 550                 555
```

<210> 20
<211> 6298
<212> DNA
<213> Ipomea nil

<220>
<221> misc_feature
<222> (1)..(6298)
<223> Nucleotide sequence of promoter region of gene encoding for protein regulating the pH of vacuoles

<400> 20

```
gatctcagtc tgtggatgtc ctagagacat tcatatttga agttgagagt tagctaaata   60

gaaaggtaaa gacaattgga tcatataaag gtgatgagta ttatgggaaa ccatcaaaag  120

ttgggcaaat tcccgtccca tttaagaaat tcctcgaatc taaaggcatt tgtgcataat  180
```

acacaatgtc aggcacacca caacaaaatg gtgtggaaga aaggtgaaat cgtactctaa 240

gggaaatggt taggagttag gtaaataatt gtacattgct tgtttcattg tggatatatg 300

cattaaaaac aacagcatac ttactcaata gggttcctag taaggttgtt ttttaaaaca 360

ccttatgaac tgtggacaag aaggaaacct agtttgagac atcttcacat tcagggttgt 420

caagctggag tgatgatata taatccacat gaaaataaat tatggatatc cagaaccatt 480

aatggttatt tcattggata tccagaaagg tctaaaggta catgtttatt gtcctaatca 540

taagtacgag gattgttgag tctggtaatg ctcgcttcat ttaaaatggc gaagtcagtg 600

ggagtgtggg agctcgtaat gttaaaatta aggagtcatt gatggttcta gattcatcaa 660

gtgatccttt tttcctgttg ttgttcctat tgttgcagtg cagtgtagcc ttatggaaat 720

actttggaac aacagcaact agatgctcaa attccacatg aggaagctat tgtaaatgaa 780

gatgaggttg aaactcaaga tgatgatcaa gtgaaatctc agcaggaagt gacattaagg 840

aggtctacta tagatagaaa agatcaacca ttcttgatga ctatattgtt tatacacttg 900

agcattgata attatccagt ctcatttaac caagccatac aggataataa ttctccttga 960

ggattatttt ggttttggtg gctcattctg ttcttgagct ccaccaaatg gttgttaaaa 1020

ttacctttct gaatggtaaa ttaaaagagg aagtatacat ggattagccg taaggcttca 1080

tggccacagg aaaggaaaat ctggtatgta gattgaagat gtcgatctat ggattaaaac 1140

atgcttctag acaatggtac ttgagattgt catttggttt tgtagagatc actgttgatc 1200

ggtgtatcca cataaaggtt taatggaagc aagtttgtaa tcctagtatt acatgttaac 1260

gacattcttc ttgctgctaa taataaaagg gatgttgcgt gatgttaagg aatagctttc 1320
taagaacttt gaaatgaagg atatgggtga gacttcatat gtgattggaa taagaaatat 1380

tccgtaatag atcacatggg attttaggtt catcctagaa gactcacatt aacaaagttt 1440

tagaaagata caaactggaa atctgcaaag agggtcctgt gatacttaca aggcactaag 1500

gaccacatgc tcacctataa aatgaacgaa taacctagag gttataggtt attcggattc 1560

agactatgcc ggatgtttgg atacccgaaa atccacattc gaatatattt ttccacttgc 1620

tcgtggagca atatcttgga tgagtgtgaa ggagcctgtc attgctactt ccactataag 1680

ggcagaattt gtagcatgct ttgaggctag tagacactat aaaaattggc tgccttgtgc 1740

caacatcatt tgcatccagc tataagcatc tccatttttcg aacatcattc gattcttata 1800

gctggatgaa gatgattcac tgcactttgg ttgcatattg aagctgcgat tgctatcgaa 1860

aacaaataac ctatctatat aaaacaaacg acttagattt agggaataag aaggaagata 1920

ctttttttaa aatcccaaaa ttacctttta ggtttgacct gcaaataaca ctttaagatc 1980
aaatcagata aaatgtcata atcaatgatc aaattgaata attttagtag tcgaggatca 2040
aattggtaaa atccccatag tcgagggact aaaccagtaa ttttctcgcg tttgaacgtt 2100
tgtccgaaaa ttggcattag cgatagctta attgagtttt tcaattctct aatttttaa 2160
attttgtttc ttcataaaat ccttcacttt ttcactttgc taatattttg ccgaatttat 2220
aatatttcca atttctaaag tagcagaacc ccagacgttg aactgccaat ttttttttt 2280
gtttttgttt tttgtttttt tatttcctta tccctccacc tcattttgaa gttaattatt 2340
attattaatt cattaatttt taaaatagag agactgcatt aacacaaaat tagccaatta 2400
ggtagcagaa ttaaatttaa acaaacaagt tggtttaatg taatttttgt caatttaatt 2460
tctctatttt tggacaaaaa ttaggtagac ttattaaatt aaataaacat gtttgtttaa 2520
ttttacttct tctacctaag tttgtgtcaa ttcagtctct ctattttata aattaatgaa 2580
tggttaaata taacttataa gtgcattgtg tccaaatgat cacaagagtt aggccaactt 2640
ctttttttcat ataggtgatt ctttttttcga gtattacgta cacttcagtc ttgtcaacta 2700
acacttagaa tttagttgtc attttcgaac ataggtgtca actaagtttg gtatccacta 2760
tatagcacat gtattccaag agatttaatc tcattcatca tgacaacttc tctaccaatt 2820
ctttgctcaa tcttttagtt agcgaattcg ctatattatc ctataacttt cagtatagtc 2880
aacaaaaata aaatgtattg caagaaacta tttaatagtg tatgttatgt atatgtccta 2940
tatgtctaga cttaccgtta tacatattac taatttacta tatgtccttc caattgcgaa 3000
ttgactatcg taatgcatac atattggaga tatatatttt ttctaggggt aaatgcaggt 3060
tggatcgacc cattaggcct gccccaacgc aaacttttt tgtcgggctt ttgcggaccg 3120
gcttgcgggt tagaaaatac acagcccaag cccgtccatg cgggctcgcg ggccttattt 3180
caaaaaaaaa aaaaatacta cgtattattc tattatttta tattcaaata gtctaatata 3240
aataaataaa aaaatcgtgt ttgaaaatta ctttttttt tatatatatt ttttaaaatt 3300
ttaatgttat atacgaagtg tgtgtaatat atatatatat atatatatat atatatatat 3360
atatatatat atatatatat attatttata ttatttatat ttatgtttat atttaaatac 3420
gggcatggct cgtcggctgg tccgttaggt ccgctctttt gtaggccatt tttttgtgtg 3480
accctaaatc gtctcaccgc gggacaagta tagggcagct tgcggacttc ggtccatttt 3540
gacatatata tatatatata tatatatata tatatatata tatatatata tatatatata 3600
tatatatata taacattaaa atttaaaaaa tatagatttt ttttaaacat gaaaaaaatt 3660

aatattattt atattaaatt atttgaatat aaaataaata atacttttt atagcttgcg 3720
ggctggtcca taaaagcccg taaaaagaat acgttggggt tggcctaatg gaccgatcca 3780
acccgcattg acacccatag gaaaaacatc tatctccaat ttgtatgcat tacaatagtc 3840
aatttgcaat tggaagagca tatagtaatt agtaatatgt ataacggtaa gtctagacat 3900
attgaacatg tacataatac tattaaatag cttcttgcaa taggttttat ttttgttgac 3960
tatacatgaa gttataggat aatatagcga attcacaaac taaaagattg agcaatgagt 4020
tagtagaaaa gttgtcatga tggatgagat taaatctctt ggaatacatg tgctagtgga 4080
taccaaactt agctgacacc tatgttcgaa aatgacaact aaattctact acgtaagtgt 4140
tagttgacaa agtagagtgt actgaatact cgaaaaaaga atcagttatg tgaaaaaaaa 4200
aagttggcct aactcttgtg atcattttag acacaatgca cttataagtt atactagtat 4260
tttttatgcg cgatgcacaa aaaatagttg cacaatatta atacattata ttaaaatttt 4320
aaatttattt agattttaga tatttaaatt gttctaacta ataatactaa taataataat 4380
gtaaataatt tttataaatt tcagatttat atttaggtaa taattaacat ataactcaaa 4440
tatataatgt gtatatatta ttattaaggg aaaatgacac ttttttccc tgagttatat 4500
accacttttt ttcccttgag ttatttaagt ggctcttttc cccctaaaat gttaaatgga 4560
catattaacc cttaaaataa atatttcaaa taaatatttc atttattttt cttctctaac 4620
aaattattac tataatttgg ttcaaaccaa acagatacta tagcaaccaa accaaaatat 4680
tccaattaca atttagaatc aaaacgcgat atttaaagtt tattaaaatt gcaaatcgga 4740
atggtcggtt catgttccga actgaaaaaa taaaatacat ttattgttga atttagacta 4800
ttttaaaata aaaaataaaa caaaatttta aaataaagac ggttcaaaat cgcgaaccga 4860
atccggaacc gccggttcac ggttcatgat ccagtttttt tggttcataa aatttaataa 4920
attgaaatct aaatattgga ttctagatct gaatcataac cgaaaacttt taattcgatt 4980
actatagtgt ccggttcagt tcgaaccgaa ccgtggtcat tgctacatat acacaataat 5040
ttgttggaga aattaaataa ataaaatgtt tactttaagg gtagaaatgt caatttaata 5100
tttcgggggg aaaaccacca cttttaaatt aattgagggg gctaatgtgc ttatataaat 5160
ataattgagg ggaaaaagtg gtataagtat ataacttagg gggaaaaaat gtcattttcc 5220
ctattattaa tgaagaagat aagaaaatat atggtgaatg catgtgcctt tatagcataa 5280
tgtacaaaaa aaacttaacg aaaaaaacaa acataaataa ggggtataac tttcattcac 5340
acttattatg tttttagatt agatttaacc atacatgcat taatttgtaa aatagcgaga 5400

gtgaattaac acaaaattag gtagaagaag taaaattaaa caaacatgtt tatttaattt 5460
aacaagtcca cctaattttt gtccaaaaat agagaaatta aattgacaaa aattacatta 5520
aacaaacttg tttgtttaaa ttgaattctt ctacctaatt ggctaatttt gtgttaatgc 5580
agtctctcta ttttaaaaat taatgaatta ataataataa ttaacttcaa aatgaggtgg 5640
agggataagg aaataaaaaa acaaaaaaca aaaacaaaaa aaaaaaattg gcagttcaac 5700
gtctggggtt ctgctacttt aaatactgat aggagagttg tcgttcattt tacaagtatt 5760
aaggatgtac acgtattgag aatgtaggct acagaaattt tcagacagat agatacataa 5820
atccgtataa tagagacaga gaaacagaaa aagagagagt cacgttaatc ctgagatttt 5880
cctccatttg tctgaagctc ttcatccttc aacactaccc ccacatctca cctttcaagg 5940
tccaatcttt atcattcatc tttaatttcc agctctatct tgggatttgc atgtaaattt 6000
tatttatttt tcgggtttct gtttccgatc ttatgctttt gttccaaagg gtatttgatt 6060
tcatatatta tgagttttgc atgcattttc tcttttgtaa aatgaaagaa aatttgagat 6120
attggtgggt ttgatctgaa agtttgtttg tttgcagtga tttgtatgtt ttcgggaggg 6180
attggaatgg gcaacccgga tatgtgaaca gaaaccacga cattgggaaa agatttattg 6240
caaaaattgt tttgattgtt ttggattttg tggtagaaaa aggggaagaa caaaaatg 6298